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SPINAL CORD REGENERATION IN THE RAT*

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INTRODUCTION

REGENERATION in the central nervous system of non-mammalian vertebrates is generally accepted (Lorente de N6, 1921; Percy and Koppányi, 1924; Detweiler, 1926; Hooker, 1932; Tuge and Hanzowa, 1937), but in mammals, regeneration is said not to occur or to be abortive (Lee, 1928; Cajal, 1928; Spatz, 1930; Rossi and Gastaldi, 1935). The more important factors in this non-regeneration are held to be (i) the absence of "embryonic" cells to help restore the gray matter (Spatz, 1930; Carlson, 1924), (ii) the lack of Schwann cells (Spatz, 1930; Yamada, 1906-8) and the lack of neurotropism and growth pathways (Cajal, 1928 for discussion). The more recent work on mammalian central nervous system regeneration has been largely concerned with rat fetuses—which should have capabilities for regeneration between those of the lower vertebrates and of the adult mammal. Gerard and Koppányi (1926) and Gerard and Grinker (1931) transected foetal rats in utero or just after birth. They found considerable return of function, but could present little certain evidence of anatomical restitution. Completely negative anatomical findings were reported by Hooker and Nicholas (1927, 1930) and Nicholas and Hooker (1928) after transection with the cautery, but they did find much return of function, attributed to the transmission of impulses by collateral pathways and to mechanical pulling of tissues to initiate reflexes past the cut. About this time, however, Migliavacca (1930a, b) reported two cases of anatomical and physiological regeneration in foetal and newborn rats. In their review of mammalian regeneration, Rossi and Gastaldi (1935) attributed this anatomical regeneration to regrowth of spinal roots and to possible incomplete section of the cord, leaving the return of physiological function to be explained by the collateral transmission of Hooker and Nicholas.

Only one paper on regeneration in young or adult mammals has appeared since 1928 (for reviews up to this time see Lee, 1928, and Cajal, 1928). Marburg (1936) found no regeneration in dogs with one or both lateral columns cut. The bulk of work has been done on rabbits, cats, and dogs. It had been noted by Gerard and Koppányi (1926) that spinal cord section in young adult rats was invariably followed by bladder disturbances, edema, and early death. Ssamarin (1926) abandoned the use of rats in his spinal cord studies "da diese Tiere die Schnitte nicht vertrugen und zugrunde gingen" (p. 374). The present work was undertaken to maintain, if possible,

* The present investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

chronic adult spinal rats, to elucidate the factors involved in non-regeneration of adult mammalian spinal cord, and to attempt to activate the abortive processes of regeneration.

The distribution of the anterior spinal artery of the rat (the chief source of blood for the spinal cord in this animal) was first investigated so that a level of section could be chosen which would avoid anemia of the several stumps (the effects of spinal anemia are well known—Gildea and Cobb, 1930; Tureen, 1936, 1938). It is probable that the "isolation dystrophy" of the caudal stump in spinal monkeys (Sherrington, 1906) is largely due to disturbances in vascular supply.

METHODS

A. For study of the anterior spinal artery, adult rats of both sexes, submerged in warm water, were perfused through the heart with warm 10 per cent gelatin colored with trypan blue (plus 1 per cent thymol to prevent bacterial action) after the blood had been washed out with 25 cc. of Ringer. The entire body was then left over-night under cold running water or in an icebox, until the gelatin hardened. The cord was exposed from the ventral side, and the distribution of the artery and its tributaries noted.

B. Spinal cord section was performed on young (3-5 weeks) albino and hooded rats of both sexes. Under ether anesthesia, the animals were placed belly down, tied by the fore-legs only, and the skin on the dorsum extensively shaved and cleaned with alcohol. A midline incision was made from the middle of the scapulae to the last thoracic vertebra; bleeding from the infrascapular fatty tissue stopped with hot moist cotton, the laminae of two or three vertebrae removed, and, after further haemostasis, the cord severed with a fine scalpel (Bard Parker No. 11) or fine-pointed scissors. In the early experiments, completeness of the cut was insured by drawing the knife blade through the cut a second time, feeling the bony vertebral canal on all unexposed sides. Later it was found that tension on the tail at the time of sectioning favored a complete cut with one knife stroke and, since the cut ends separated 1 to 3 mm., left no uncertainty of the completeness of section. After hemostasis, either the ends were brought back into apposition or other tissues placed in the gap. In one series, bits of dorsal musculature were inserted (series RSR); in another, mashed or intact embryonic brain or spinal cord (series RT); in a third, segments of degenerated sciatic nerve (series RNC). The muscles, dorsal fascia and skin were then sewn in separate layers with silk. No dressings were applied. In a subsequent series of spinal rats (suggested by Dr. Lorente de N6) partial sections were deliberately made in order to eliminate any injury to spinal roots. Lesions ranged from small stab wounds in the dorsal columns to lateral hemisection, at levels selected to avoid dorsal roots.

The embryonic nervous tissue was obtained from 3-4 cm. rat fetuses, removed from the mother (ether anesthesia) after the spinal cord of the recipient was exposed, and exhibiting good reflexes at this time. To obtain brain tissue, the head was cut off and the cranial contents squeezed out. More intact cord was obtained by cutting it from the spinal column with sharp scissors. The sciatics were taken from young adult rats, 2 to 4 weeks after section at the exit from the greater sciatic foramen. Adequate *asepsis* was attained by soaking instruments and skin in 70 per cent alcohol, since no primary wound infection occurred. It is essential that bleeding be well stopped before the wound is closed. The blood lost is usually quite small, but a post-operative intraperitoneal injection of 5 cc. of warmed saline was regularly given.

Operated animals were kept in solid floor cages on sawdust, since the paralyzed extremities suffer injury in open wire meshes. For 10 to 15 days, until the bladder became automatic, urine was expressed three times daily by pressure on the lower abdomen. The animals were frequently tested for sensation and reflexes and their spontaneous movements and activities observed. Finally, under ether anesthesia, the brain was exposed and stimulated (Harvard inductorium, 3 volts in the primary, secondary at 7 to 9 cm.). The animal was then decerebrated, the anesthetic discontinued, and the cerebral peduncles stimulated directly. Finally, the animals were perfused through the heart (intestines clamped off) with 20 cc. of Ringer followed by 100 cc. of a mixture (Bodian, 1937), of 80

per cent alcohol (90 cc), 100 per cent formol (5 cc), and glacial acetic acid (5 cc).

The spinal cord was then exposed from the ventral surface and the region of section, including 3 to 4 segments on either side, placed in the same fixative for 24 to 48 hours, dehydrated with cedarwood oil, and embedded in paraffin. In a few instances, when the cord ends showed poor continuity or were attached to the periosteum, the cord and surrounding bone were removed, fixed, and decalcified in 5 per cent nitric acid in 70 per cent alcohol for 6-9 days. Sections were cut 20 to 25 micra thick in either frontal or sagittal planes, and mounted serially for staining with protargol by the silver-on-the-slide method of Bodian (1936, 1937). Every 10th or 12th section, however, was mounted serially on a separate slide for staining with toluidin blue and erythrosin or with Ehrlich's hematoxylin and eosin. In some cases, formalin fixation was used and blocks of cord above and below the lesion were sectioned transversely and stained as above or with an iron-haematoxylin method for myelin sheaths (Mahon, 1937). Animals dying before termination of the experiment were discarded.

RESULTS

General observations

Blood supply. In the rat, the anterior spinal artery does not arise from the vertebrals, but rather is formed by the confluence of branches of a number of vessels ascending along dorsal roots (Fig. 1). From C1 to T12 it is built from caudal and cranial branches of 6 to 10 such arteries, irregularly distributed, and usually coming more from the left than the right. At T12, T13, or L1, a large entering vessel anastomoses through a fine connection with the thoracic portion of the artery, then curves caudally to become the lumbo-sacral portion. (In 5 males and 1 female, this vessel arose from the right; in 3 females, from the left.) Usually no vessels enter the artery below this large one. The anterior spinal artery joins the usual branches of the vertebral arteries at its cranial end; caudally it loses itself in terminal twigs.

The cord was cut above T12, to avoid the large blood vessel. Section below T13 uniformly led to degeneration of the caudal stump of the cord with atrophy of the hind-limbs, and failure to regain bladder control. (In the dog, bladder automaticity has been held to return in the absence of the lumbar cord, Goltz and Ewald,

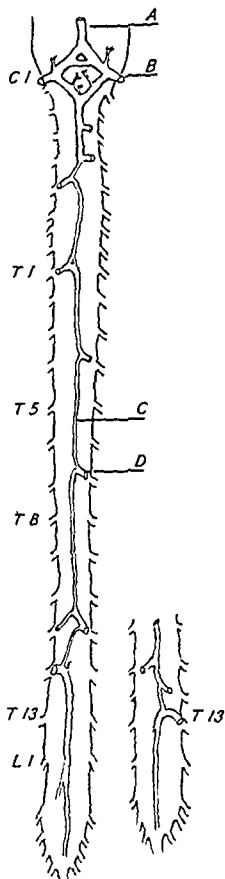


FIG 1 Diagram of the anterior spinal artery of the albino rat, based on 5 males and 4 females; the most common variation, found in 3 of the females is given in the inset. A, basilar artery, B, vertebral artery, C, anterior spinal artery, D, radicular branch of spinal ramus of intercostal artery.

1896; but this has recently been denied, Morvan, 1936). Section above T5 interferes with fore-limb and respiratory movements. In 23 of 28 fully studied cases the section was between T6 and T9. There is no correlation between the level of cut and bladder control or length of survival.

Bladder control. In females, urine is easily evacuated by squeezing the abdomen. In males, the tenacity of the internal sphincter and the occasional occlusion of the urethral orifice with sawdust or semi-solid secretions makes necessary considerable pressure; enough to rupture the bladder in extreme cases.* Expression of urine 3 times daily provided adequate emptying and prevented cystitis, pyelitis and hydronephrosis. One emptying a day was wholly inadequate; two might suffice when evenly spaced. The quantity of urine obtained at one time was roughly recorded as: + + +, 2 to 3 cc., the usual accumulation of 8 hours; +, 0.5 cc. or less; and 0. Occasionally as much as 5 cc. were expressed.

The bladder became automatic after 8 to 12 days (most commonly 9) in both sexes alike, unless degeneration of the caudal cord segment prevented any return or intercurrent illness delayed it three weeks or more. Automaticity was considered present when the three urine amounts expressed in a day totaled 5+ or less instead of the usual 9+. Well-formed fecal pellets were regularly dropped at least by the second day, so intestinal function offered no nursing problem.

With adequate bladder emptying, the gangrene and edema previously encountered (Gerard and Koppanyi, 1926) were practically eliminated. The coincidence of bladder dysfunction and edema is well shown in rat RNC 1 in which bladder control was present from the 10th day after operation. Five months later the left leg became edematous and the bladder was found to be distended and the penis engorged due to a caseous plug in the urethra. This was squeezed out and urine regularly expressed for a week, after which automaticity was reestablished and the edema entirely gone. As a further check, in four animals the postoperative bladder evacuation was omitted. All developed edema and gangrene and three died within a week.

Of 47 rats operated on (16 males and 31 females), 15 died within two weeks from bladder disturbances, etc. The rate for males was much higher for this period only. Late deaths were mostly from pneumonia. Once past the early hazards, the life of the operated rats is potentially the normal span, and the animals grow at a normal rate. Those animals living 14 days or less have not been examined in detail. Usually they died at night and the spinal cords could not be salvaged.

Reflexes. The reflex activity of spinal rats is considerable. Such animals make excellent demonstration material. An ipsilateral flexion reflex can be elicited within 1 min. after the animal awakens from anesthesia, long before proper coordination of the anterior part of the body has been regained, and consists of a simple withdrawal upon pinching the foot unaccompanied

* This is not necessarily fatal.

by after-discharge or spread. Withdrawal of the tail in response to pinching it appears on the second or third day after operation. Later (3 to 7 days) the same stimulus also causes stepping—bilateral alternate rhythmic hip and leg movements—which eventually considerably outlast the stimulus. Crossed responses are added to the uncrossed also on the 2nd or 3rd day. Pinching the toes causes ipsilateral flexion and contralateral rhythmic “pushing-away” movements, similar to the stepping response, but directed towards the stimulated foot. This defensive reaction becomes more and more marked and in later stages appears almost voluntary. In a few cases, it could also be elicited by traction on the crossed leg. Simple crossed extension appeared only in isolated instances. A complicated reaction appeared in a number of cases, about 10 days after the operation, when the animal was lifted by the chest. The caudal segments and hind legs were stiffly extended, with toes flexed, for a few seconds and then the body relaxed or “curled”, with flexed toes, hip, and knee, and adducted thigh, so that the legs were closely pressed to the abdomen. This defensive shrinking position was sometimes assumed without the initial “standing” posture.

During the first week or ten days, the animal drags itself around with fore-limbs and the flaccid hind-legs are passively stretched behind or flexed as the body turns. Later, however, walking movements of the hind-legs, mainly alternate flexion of the hips, appear irregularly, usually after the legs have been dragged passively for some distance. Scratch reflexes develop between the 11th and 14th days. A standard undirected response can readily be obtained by stroking the lower abdomen or a number of other regions but never involves the leg across the midline from the stimulated skin. A mass reflex is but rarely provoked by pinching the toes and never by other forms of stimulation (e.g., stroking the abdomen). “Spontaneous” movements, stepping when the animal is lifted and scratching with no apparent cause, are occasionally seen.

Physiological recovery

The reflex activity of the hind-quarters described above represents the final equilibrium of the true spinal animal. No sensation returns. Thirteen animals acquired additional motor and sensory capacity, associated with anatomical restitution. A number of tests were used.

When placed on a vertical wire mesh, spinal animals climb or, usually, descend by means of the fore-legs. The hind-legs drag passively, are not placed, and usually catch in the wire and make the rat fall. When suspended by the tail, the hind-legs, in the spinal rat, hang passively with lax toes and do not move; while in the normal, the legs and toes make searching movements and, if a support is encountered, grasp it and right the animal. When placed on its back, only the fore-quarters of the spinal animal perform righting movements and the inert rear often prevents a successful turning over. On the sensory side, the forward transmission of pain impulses (pinching tail) can be signalled by struggling of the fore-quarters or vocalization—

never observed in the spinal animal. Hind-legs remain where they fall or have been put, indicating absence of proprioception. Finally, electrical stimulation of the peduncles (and rarely of the cerebrum) of intact animals evokes generalized contractions; but in spinal ones all response stops completely at the transection level. Even with much stronger stimuli (secondary at 3 cm.) no "spread" beyond the lesion occurs and, during stimulation, the vigorously contracted front half adjoins the flaccid motionless rear.

The animals showing recovery performed somewhere between the normal and the spinal rats on these tests. In walking, toe movements contributed to the hind-leg action; and toe flexion and extension sometimes occurred while the animal lay at rest. A peculiar hopping of both hind-legs alternated with stepping movements in three rats and enabled them to progress indefinitely. Placed on a vertical mesh, eight animals used one or both hind-legs actively in climbing, even at times in descending, with seeking and some placing movements. These appeared during the second post-operative month. After the 4th or 5th week, when any of the recovering animals was hung by the tail, the hind-legs were held stiffly outstretched except for occasional rhythmic and alternating seeking movements. Effective grasping never was observed. In one animal, the left hind-leg participated in righting movements; and it readily regained its feet when placed on its back or either flank. Pinching the hind-legs or tail evoked struggling movements of the fore-quarters but no squealing. The hind-legs and toes were placed with more or less accuracy in seemingly voluntary movements and were drawn into a proper posture when displaced, indicating active proprioception. Finally, stimulation of the peduncles with moderate shocks (secondary at 7 cm.) caused movements caudal to the transection. Simple flexion or extension and rhythmic stepping or thrusting were elicited on either or both sides depending on the rat and on the exact site of stimulation. Sometimes a marked after-discharge persisted behind, but not in front of, the plane of section, so that the fore-quarters had relaxed at the end of stimulation while a hind-leg, for example, continued to step for 5 to 10 seconds.

Anatomical findings

Three rats of a preliminary series showed incompletely sectioned cords. These are satisfactory controls, for in all of them some voluntary movement was present at least by the second post-operative day and coordinated movement and sensation rapidly improved until nearly or quite normal. Similar rapid and complete recovery occurred in the later series of animals with hemisections or lesser lesions. In all other animals the cord had been completely severed and a connective tissue scar bridged the cut ends.

Cysts were often present, one on either side of the central scar, particularly in cases in which a tissue had been placed in the cut. They sometimes had thick walls containing normal nerve cells, which thus were close (in one case within 175 micra) to the scarred region (Fig. 2). In the scar were many infiltrating leukocytes and connective tissue bundles, which usually ran

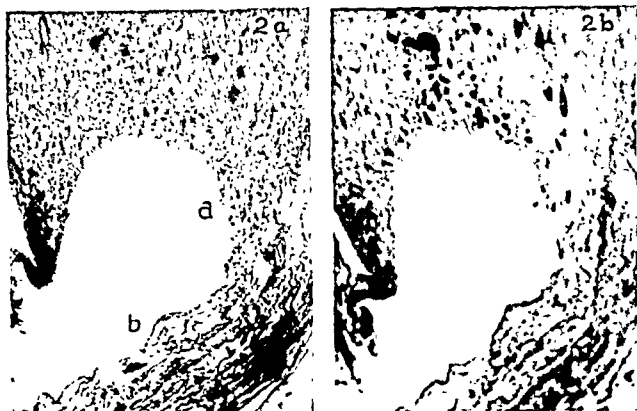


FIG. 2. Fig. 2 through 9b are photomicrographs of sagittal sections of spinal cords of operated rats. Fig. 2b and 3 are from sections stained with toluidin blue and erythrosin: the remaining are from sections stained with the silver-on-slide method of Bodian. 2a Craniad stump of spinal cord. The dorsal half of the cut region was occupied by a cyst; a, clump of normal cells; b, connective tissue scar with new fibers arising from ventral grey matter; $\times 80$. 2b. Section adjacent to that of Fig. 2a. Note deeply-staining Nissl substance in cells around the rim of the cystic area: note also apparent absence of nerve fibers; $\times 80$.

transversely across the base of the stumps or the cysts. In general, the scarring and the degeneration changes in the white matter were as described by Cajal (1928), with retraction balls, autotomy of fiber ends, axon varicosities, and the like. No extensive red cell collections, or traces of their breakdown and phagocytosis could be found.

Transplanted embryonic material in most cases did not stay between the cut ends of the cord. When it did, it either formed a dense degenerated mass or, more typically, became organized into cell clusters resembling glandular acini (Fig. 3). These "acini" were seen only following embryonic nervous tissue implantation, except in rats with partial lesions. Three of these animals showed entirely similar cell nests derived, presumably, from ependymal elements. In two cases, well differentiated pyramidal neurones developed from the embryonic implant. These small pyramids were seen in the scar

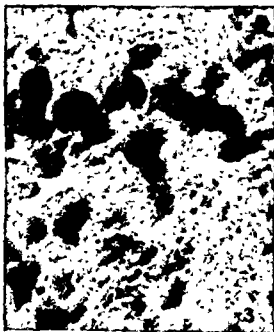


FIG. 3. Scar region with acini-like cell clusters arising from transplanted embryonic brain. Note profuse neighboring connective tissue cell nuclei; $\times 400$.

tissue, well separated from either cut stump, and their processes could be traced some distance through the scar. These neurones were quite distinct from normal cord elements and definitely developed beyond the embryonic stage at implantation.

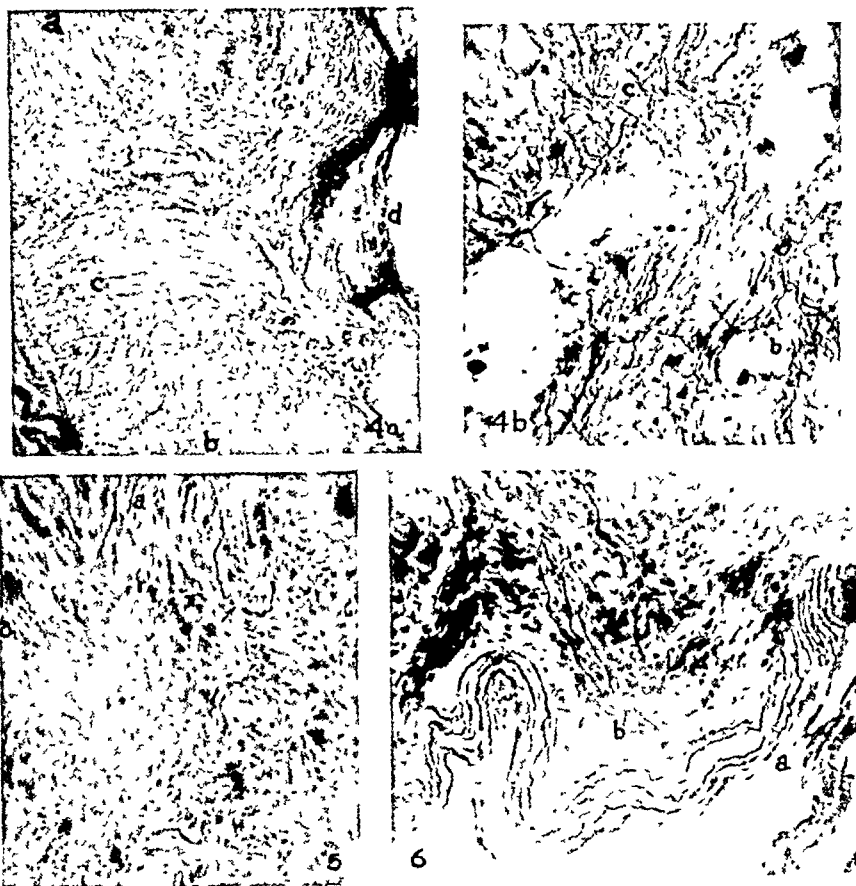


FIG. 4a. Scarred region of spinal cord, showing horizontal orientation of complete scar as compared to vertical white columns. Note nerve fibers and many *boules d'arrêt* in both stumps; $\times 48$; a, upper stump; b, lower stump; c, scarred region; d, residual hemorrhage.

FIG. 4b. Scarred region containing haphazardly running fibers arising from neighboring stump. High power of a small region of Fig. 4a; lettering as above; $\times 240$.

FIG. 5. Scar region containing degenerated sciatic nerve transplant; $\times 240$; a, bundles of new fibers running in old nerve paths. Arrow points to a new fiber curving because of connective tissue obstacle.

FIG. 6. Origin of new fibers from ventral spinal root; $\times 240$; a, ventral root; b, new fibers growing into scarred region.

Neither nerve cell mitoses nor any other signs of grey matter regeneration were seen. On the other hand, many new fibers were found in the scarred area. These were placed in confusion for the most part and paralleled the

connective tissue strands (Fig. 4), ineffectually crossing from one side to another. They followed devious pathways to avoid connective tissue bundles, hemorrhages, and other obstacles and, if no other opening was found, recurved on themselves at a 180° angle (Fig. 5), like regenerating fibers in a peripheral nerve stump. Occasionally, however, thin bundles of nerve fibers could be traced across the whole scar from one stump to the other. Some of these new fibers clearly originated from spinal roots (Fig. 6), while others as surely came from the longitudinal columns in the spinal cord (Fig. 7). Thus, there was definite continuity in one case (RSP 11) of the severed left lateral column; and in another (RT 4) of the ventral and lateral columns.

With either muscle or nerve interposed between the cord ends, the growing nerve fibers were influenced. Muscle fibers placed transverse to the cord axis prevented longitudinal nerve growth; but when the orientation of the muscle fibers was parallel to the direction of the cord, axone growth across the gap was definitely improved (Fig. 8). The nerve implants served likewise as a growth bridge and new nerve fibers followed faithfully the path laid down by the degenerated ones, even when this curved back in a semicircle (Fig. 9).

The correlation between the anatomical and physiological pictures in individual animals was excellent. Absence of reflexes and an automatic bladder was always related to a degenerated caudal cord segment. The typical spinal animals, with strong reflexes but none of the further accomplishments, possessed healthy cord stumps separated by a scar with interlaced nerve fibers not continuous between them. The thirteen rats that showed physiological recovery had new fiber bundles connecting the cord stumps across the scar and, roughly, the amounts of restitution of structural connections and functional capacities were alike. Thus, the rat (RSP 11) that used its left hind-leg in righting itself had good continuity of the lateral column on that side; and one (RNC 3) with considerable "voluntary" leg movement and good responses to stimulation of the peduncles had many new fibers (running in a degenerated sciatic) connecting the cord stumps.

Two cases of incomplete cord section, already referred to, are also instructive; for the functional recovery far outdistanced the anatomical. Rat RSP 13, had voluntary leg movement on the day after operation, and proprioception and motor control returned during 15 days, when the animal could balance itself on its hind-legs and wipe its nose free of vaseline with both fore-paws in normal fashion. Histologically, the right ventral horn and lateral column were intact, but both posterior columns had been completely sectioned. The scarred area contained many new nerve fibers, some from a regenerating posterior root, and some clearly arising from columns of the spinal cord. The return of proprioception or other sensation from the legs and tail makes it certain that some of these new nerve fibers were functioning. Rat RT 15, had the entire dorsal half of the cord sectioned, down to the central canal. Nevertheless, after about four weeks, this animal had considerable proprioception, and by two months was walking almost normally.

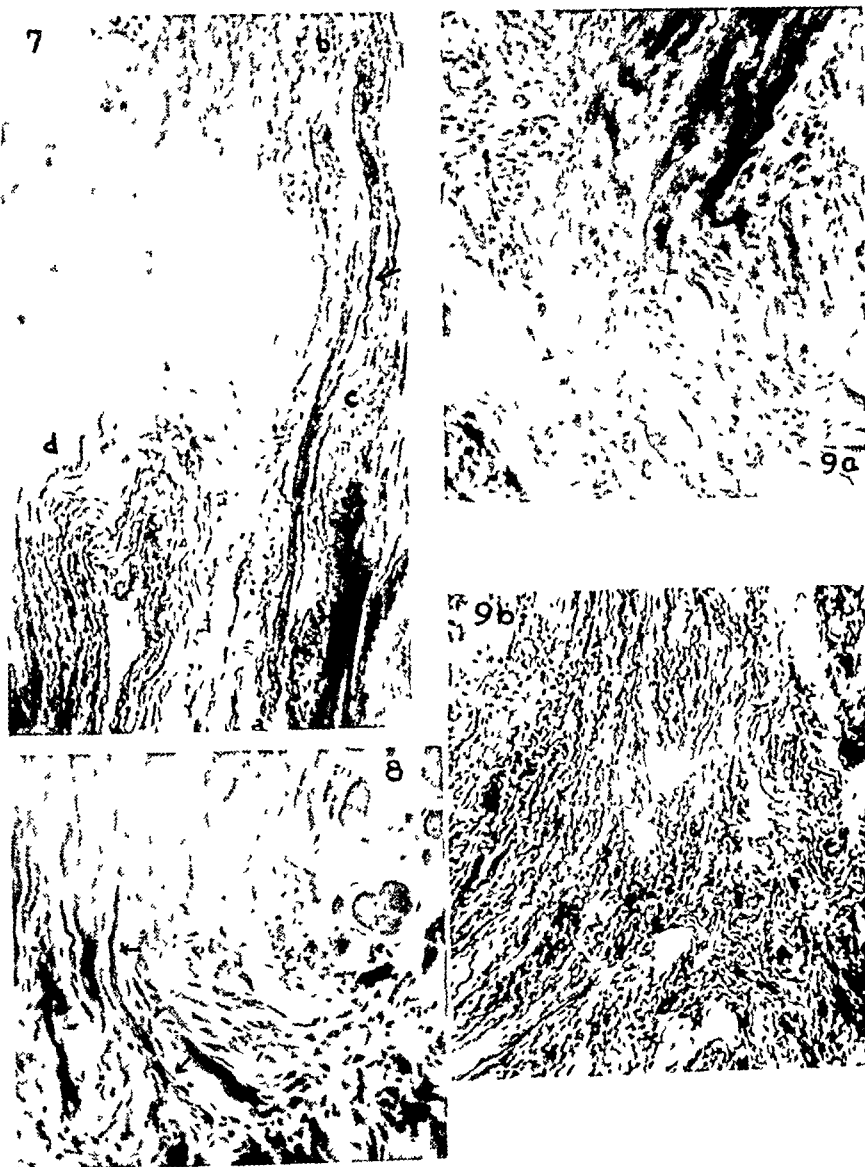


FIG. 7. Origin of new fibers (a) from lateral columns (b). Arrow points to bundle of new fibers splitting to avoid the large connective tissue mass (c). At (d) is a large group of fibers from the lower stump, recoiling at 180° . The large out-of-focus mass at the upper left is connective and degenerating embryonic transplanted tissues, $\times 80$.

FIG. 8. Orientation of new nerve fibers along longitudinally arranged interposed muscle fibers. Arrow points to nerve bundle, $\times 240$.

FIG. 9a. Regenerating cord fibers running into diagonally placed transplant of degenerated nerve. The dark material at the upper right is the transplant, in which may be seen fibers, which arise in the scar tissue below; $\times 240$.

FIG. 9b. Regenerating fibers curving with curved nerve transplant. Continuation of Fig. 9a, with fibers originating below lower left; $\times 125$.

Histologically, regenerating fibers connecting the cut ends were seen. Similar functional recovery correlated with appropriate anatomical regeneration occurred in the later series of partially sectioned rats.

DISCUSSION

The disagreement between the histological results reported here and those of some of the other workers seem to depend primarily on the methods used. Hematoxylin and eosin, or toluidin blue and erythrosin, while invaluable for studying general structure and cellular alterations, are virtually useless in tracing newly formed nerve fibers. Since, in long-term experiments, normal-appearing cells (on Nissl and silver staining) may be present within 0.2 mm. of the scar, and since the return of function might be due entirely to new fiber paths between the two spinal cord stumps, a silver stain for nerve fibers seems imperative. Migliavacca (1932) has criticized the anatomical findings of Hooker and Nicholas (1930) for the absence of silver methods. Further, serial sections in the longitudinal plane are essential for tracing fibers between stumps. The inadequacy of cellular staining is clearly shown by comparing adjacent sections stained alternately with toluidin blue and erythrosin, and with silver, for only the latter brings out new fibers wandering into or across the scar (Fig. 3 and 4b).

Silver stains in general do color connective and collagenous tissue as well as nerve, but the method used gives clear contrasts and, as checked with known nerve fibers, the neural elements can be unequivocally identified. The uncertainty of block impregnation methods and the frequent necessity for discarding sections near the surface is well known. The silver-on-the-slide method of Bodian (1936, 1937) allows uniform and repeatable staining with silver, and selection of desired sections for cellular stains. (After formol fixation, even the iron-hematoxylin method of Mahon can be used.)

On the physiological side also, much of the earlier work has been methodologically unsatisfactory. Complete cord section affords the best test for regeneration since in partial lesions, as shown above and previously (Foerster, 1930; Gerard and Grinker, 1931), extensive vicarious functioning simulates anatomic repair. Yet "blind" cord section—through the dorsum of an embryo (Hooker and Nicholas, 1927, 1930; Gerard and Grinker, 1931; Migliavacca, 1930a, b) or between vertebrae (Cajal, 1928)—entails the danger of sparing part of the cord unless, to avoid this, excessive lesions are produced. Further, direct visual proof of the section is lacking in closed operations; and a trapped hemorrhage, regularly present, forms a barrier to fiber growth. Section of the exposed cord after laminectomy has been little used and then in conjunction with inadequate staining (e.g., Yamada, 1907). Placing gauze between the cut ends (Ssamarin, 1926), while efficiently preventing hemorrhagic barriers, undoubtedly causes walling off of the ends and organization of the connective tissue in directions unfavorable for regeneration. Mechanical section with a clean sharp knife stroke causes less ultimate tissue damage than contusion, coagulation, or freezing. The elec-

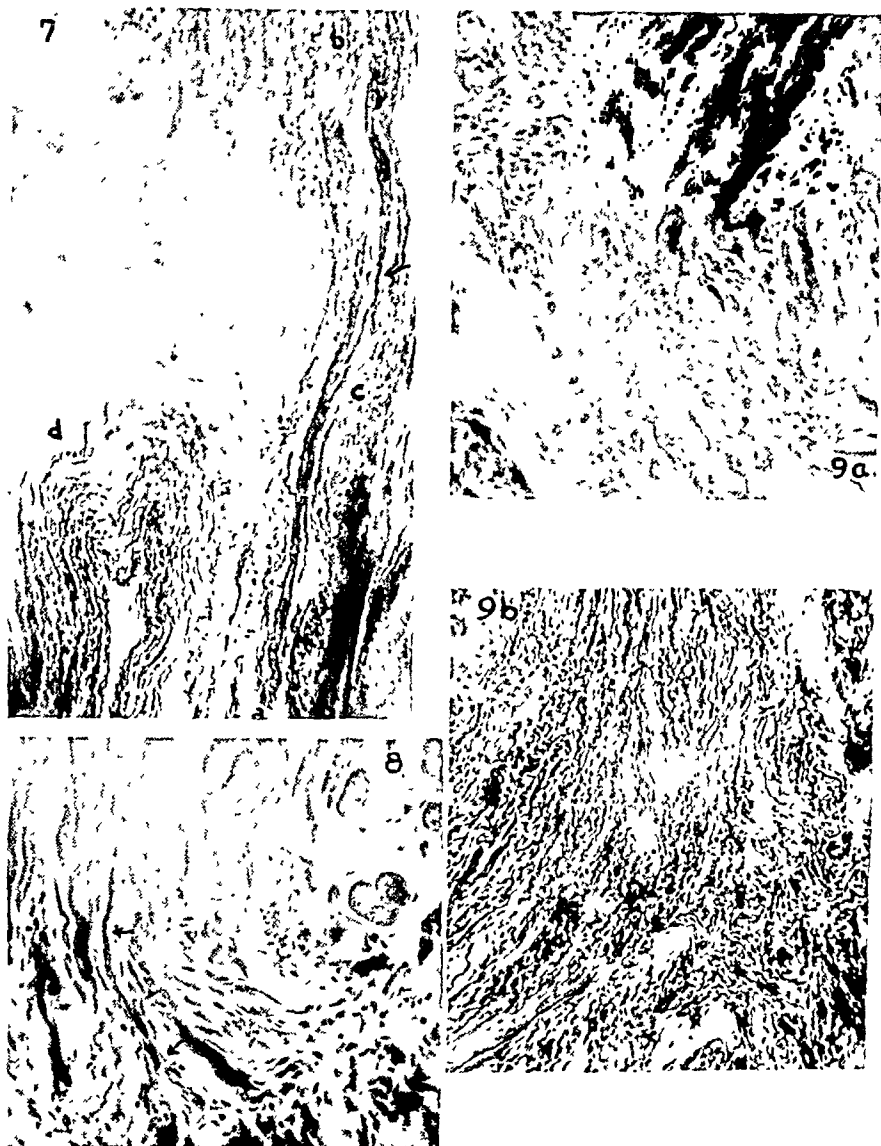


FIG. 7. Origin of new fibers (a) from lateral columns (b). Arrow points to bundle of new fibers splitting to avoid the large connective tissue mass (c). At (d) is a large group of fibers from the lower stump, recoiling at 180° . The large out-of-focus mass at the upper left is connective and degenerating embryonic transplanted tissues; $\times 80$.

FIG. 8. Orientation of new nerve fibers along longitudinally arranged interposed muscle fibers. Arrow points to nerve bundle, $\times 240$.

FIG. 9a. Regenerating cord fibers running into diagonally placed transplant of degenerated nerve. The dark material at the upper right is the transplant, in which may be seen fibers, which arise in the scar tissue below; $\times 240$.

FIG. 9b. Regenerating fibers curving with curved nerve transplant. Continuation of Fig. 9a, with fibers originating below lower left; $\times 125$.

direction. The occasional presence of bridging nerve bundles in animals that failed to show functional recovery (3 cases) is not, of course, in conflict with the above. These fibers were always sparse and sometimes originated from roots or, presumably, made unfortunate connections. The conclusion seems inescapable that nerve fibers can grow from spinal elements across a region of complete discontinuity and can then conduct nerve impulses which restore transmission across the lesion and some degree of normal function.

Some further consideration of the source of the regenerated fibers is required because the remote possibility exists of a functional bridging of ununited stumps by fibers growing from sectioned roots distal (or proximal) to the cut and reaching the proximal (or distal) cord stump. In the total cord sections, roots were cut and did indeed contribute new fibers, though the ventral roots participated but little. Dorsal and some ventral fibers usually followed the scar transversely but bundles of them could, not uncommonly, be traced longitudinally well into a cord stump, where they ended in growth cones or were lost among other fibers in the white columns. (Cajal, 1929, p. 533, had traced root fibers to the stump after 5 days but did not follow them further). Such root fibers, besides offering a possible conducting path for impulses, also might serve as trail breakers for other cord fibers later growing by their sides.

Whatever contribution regenerated root fibers may make, however, they are certainly in no way vital to cord regeneration. Even in total sections it was often possible to follow bundles of axones from a cord tract in one stump across the scar into grey or white of the other stump. This was especially clear in cases with nerve implants in which root fibers could be shown not to come into question. Further, ventral roots were rarely cut and, in the special series with partial sections, no roots were injured; yet the same new fibers completely crossing the scar were readily seen. (Some lateral herniation in these cases makes it especially easy to trace the regenerated fibers). Groups of 2 to 5 counted up to hundreds of fibers in a single partial transverse section; and, since relatively more new growth follows large lesions than small ones (Cajal, p. 510), a really considerable number must cross a complete section.

The practical success of cord regeneration (and presumably any central regeneration) is determined by the fibers growing across the lesion. Many growing axones penetrate into the scar tissue (see also Ssamarin, 1926; Cajal 1928) but more rarely do they cross it (Gerard and Grinker, 1931). To encourage this successful bridging, we tried implants into the cut of embryonic nervous system, of adult muscle, and of degenerating nerve, which might liberate "neurotropic" substances or supply a material scaffolding. The embryonic nerve cells rarely survived and then mainly as acinar clumps. Perhaps still younger embryo tissue might adapt itself and grow its own fibers to knit in with the injured cord; in two of our cases, however, definite neurone development occurred. It is interesting that Willis (1936) was able to culture almost any embryonic tissue except nervous tissue as intracerebral implants.

Muscle and nerve definitely serve as scaffolding for growing nerve fibers, which follow the direction of the implanted elements. All our best cases of regeneration had such an implant, mainly nerve which retained a favorable orientation. It should prove possible, with greater care in placing and fixing a nerve implant, to secure more regular and complete spinal regeneration.

Degenerated peripheral nerve has long been used as a bridge in efforts to encourage neural regeneration. Marinesco and Dustin (see Cajal, 1928) employed it in peripheral nerve gaps; Loez and Arcaute (1913) in sectioned optic nerves; and Tello (1911, 1924) in cut cerebral white matter. In each case, new axones grew into the degenerated paths. Celloidin blocks (Oye, 1928) and elder pith (Tello, 1924) have also been used in studies of central regeneration, with indications of axone growth. The present results seem to hold more promise of clinical application than did the early ones.

Cajal (1928) attributed failure of central regeneration to lack of pathways and of neurotropic substances; others (Spatz, 1930; Yamada, 1907) have blamed the absence of Schwann cells. Weiss (1934) has urged, especially for the outgrowth of new fibers in embryos, that fibers follow the structural paths of least resistance. Our results support this view. Individual fibers avoid obstacles and follow surfaces (a positive thigmotropism)—including blood vessels (Shirai, 1935),—and even recurve with them. That the Schwann cells are not essential to fiber regeneration is attested by the outgrowth of peripheral axones when Bands of Bugner are missing (see Weiss, 1934, and Rossi and Gastaldi, 1935) or in advance of the Schwann cells into scar tissue, in tissue culture (Harrison, 1908), and in initial growth (Harrison, 1924; Speidel, 1932). On the other hand, Schwann cells certainly aid regeneration in peripheral nerve; and in a few striking cases glial cells were seen to have formed in "bands" along regenerated central axones in a fashion analogous to that of Schwann cells in the periphery (Fig. 10).

The common failure of central regeneration is primarily due to improper pathways for the regrowing nerve fibers. This is a consequence of the early and rapid growth of glia and connective tissue to form an impenetrable mass of haphazardly arranged fibers and cells before the nerve regeneration gets well under way. This is further evidenced by experiments (Sugar and Gerard, unpublished) in which the central ends of cut peripheral nerves were led into the cerebral cortex. New fibers grow out into the cerebral tissue but are eventually blocked by a tremendous accumulation of glia and macrophages which results from the injury and the presence of a foreign body.

Regeneration depends primarily, of course, on the presence of viable neurones not too far from the lesion which must be bridged by new axones. Insufficient blood supply to the cut ends of the cord is often an additional factor hindering regeneration. The cranial stump almost always receives enough blood from the vertebrals via the spinal arteries, but the caudal stump usually depends on aortic branches for its supply. If these branches are cut, as in sections below T13, the caudal stump suffers degeneration and of course does not show repair. The choice of lumbar and sacral levels for

placing lesions (e.g., most of Cajal's) is thus unfortunate and handicaps successful regeneration.

In higher animals the meagre collateral circulation of the cord militates against regeneration. Thus, in the cat (Tureen, 1936), occlusion of the ab-

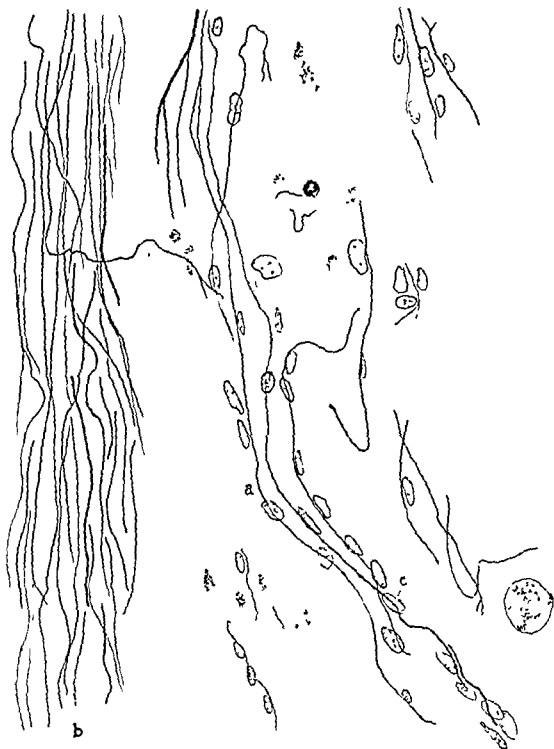


FIG. 10. Drawing of glial cell "band" accompanying regenerating central axones crossing hemisection. a. Bundle of new fibers arising in upper segment of cord with fibers of lateral column (b); c. glial cell nuclei.

dominal aorta causes degeneration of the spinal cord below the occluded level though the anterior spinal artery is intact. Conversely, paralysis from occlusion of the anterior spinal artery is well known in man (Zeitlin and Lichtenstein, 1936). In the rat, however, section of the anterior spinal artery

per se (cord lifted out of the way) is not followed by permanent damage to the cord, and the same is reported for the goat (Hunter and Royle, 1924). Poor blood supply probably accounts for the rather frequent failure of spinal humans (Riddoch, 1917) and monkeys (Sherrington, 1900) to develop proper spinal reflexes. Indeed, as blood supply is essential for normal neural function (Gerard, 1938), so it must influence regenerating vigor; and quantitative variations in vascularity may help control the degree of regeneration at different loci in the nervous system and in animals of different age and species.

As a spinal mammal, the rat seems to occupy a position on the phylogenetic scale slightly above the rabbit (Hinsey and Cutting, 1933) and definitely above the opossum (Hinsey and Cutting, 1936), but distinctly below the dog and cat (Sherrington, 1900) and the monkey (Hinsey and Markee, 1938). Such a rating is based on the time required for regaining spinal reflexes and bladder automaticity, and on the degree of spinal shock. Shock is almost transitory in the opossum, but enduring in the carnivores and higher animals, thus paralleling the degree of cephalization (Hinsey and Cutting, 1936; Fulton, 1938). It also varies inversely with blood supply; and work is in progress on a possible anemic factor in its production.

SUMMARY

1. Spinal rats (cord section between T5 and T13, with tension on the tail causing the cut to gape 2-3 mm. and insuring a complete lesion) will live indefinitely (8 mos.) with moderate care.

2. Edema and gangrene of the hind-legs (as well as hydronephrosis and hydroureters) are the main cause of early death and depend directly on urine retention. Manual expression of urine 3 times a day during the period of returning bladder control, 7 to 10 days, eliminates this difficulty.

3. Flexion reflexes are present immediately after section, crossed responses appear after 3 days, scratch reflexes after 11 days. Complex and fractional responses of hind-legs and tail develop, but with no sign of coordination with the front quarters.

4. By one month, the typical spinal animal has reached a steady level of behavior and fails various tests of sensation and voluntary motor control. At the time of sacrifice electrical stimulation of the cerebral peduncle does not evoke motor responses below the level of section. Cell stains show normal neurones close to a complete transverse scar and silver methods reveal new axones entering and becoming tangled in the connective tissue of the lesion.

5. In some cases (13 in all), after four weeks of typical spinal performance, further sensory and motor recovery occurred, including voluntary climbing, walking and hopping movements, and placing and seeking based on good proprioception. At sacrifice, stimulation of the brain stem produced hind-leg movements. Silver stains showed a complete scar bridged by bundles of new axones passing continuously between cord tracts on either side of the lesion. The fibers entering and crossing the scar ordinarily arise

from both the cord tracts and spinal roots, but when the latter are excluded physiological recovery still occurs.

6. The most marked recovery was in rats with properly oriented nerve or muscle implants in the cord gap. Recovery was poor with embryonic brain implants, which degenerated, formed acinar cell stumps, or developed into small pyramids.

7. Spinal neurones with adequate blood supply start to regenerate cut processes. These fibres grow along structural pathways like peripheral nerves and using bands of glial nuclei when possible, but are mainly blocked by glia and scar tissue running transversely across the cord. When they successfully cross a scar, restoring anatomical continuity, nervous transmission across the lesion and coordinated function also return.

8. True anatomical and physiological regeneration can occur in the rat spinal cord. This is aided by an implant of degenerating sciatic nerve. Such implants may find clinical application.

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PALEOCEREBELLAR INHIBITION OF VASOMOTOR AND RESPIRATORY CAROTID SINUS REFLEXES

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INTRODUCTION

THE PROBLEM of cerebellar function is still controversial. It is agreed, however, that the cerebellum acts in relation to volitional movement and therefore may be considered a center of *somatic* nervous activity. The possibility of an action on the *autonomic* functions has often been considered. A complete review of the literature on the subject was given in a previous paper (Moruzzi, 1938). One need only recall here that this action has been conceived of in two different ways: (i) as an influence of the cerebellum on voluntary muscles through the intermediation of the autonomic nervous system (Camis, 1913; Krestovnikoff, 1926); or (ii), as a direct cerebellar action on visceral organs. The object of the present work is to investigate the latter possibility.

This second relation was particularly stressed before the work of Luciani. Afterwards the theory was almost abandoned, but has been taken up again recently by different authors and notably by the Orbeli School in Russia. Experiments so far carried out on this subject have not appeared convincing perhaps owing to the fact that the results were often contradictory and open to technical objections (spread of physical stimuli in the excitation experiments, secondary lesions of neighboring centers after total or localized cerebellar extirpations, indirect effects caused in a reflex or purely mechanical manner by changes in the activity of the striated muscles). In any case these experiments have played little part in the formulation of recent doctrines on cerebellar physiology (Miller, 1926; Van Rijnberk, 1931; Bremer, 1935). Conclusions drawn from them have been formally discarded by other authors (Dusser de Barenne, 1937; Karplus, 1937). Nevertheless Fulton in a recent review (1936) insists on the fact that in the premotor area of the cerebral cortex the somatic and vegetative functions have a focus of common integration; in view of the close relation between the cerebral cortex and the cerebellum, it would not be surprising if "further investigation would lead us to a conception of ataxia and asynergia in autonomic regulation similar to that now held for cerebellar disturbances in the somatic sphere."

We have recently (1937, 1938) examined the function of the paleocerebellum in relation to a fundamental activity of vegetative life, the nervous regulation of vasomotor tonus. These experiments, which were carried out on precollicular cats, were concerned exclusively with the excitable area of

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the paleocerebellum. This area coincides, as Bremer (1922) has shown, with that part of the paleocerebellar cortex which forms the site of termination of Flechsig's and Gower's tracts and thereby receives the name of "spinal story" (Ingvar) or "Pars somatica" (Ariens Kappers) of the cerebellum. In our experiments, therefore, we decided to determine whether a weak faradic stimulation of the excitable zone of the paleocerebellar cortex, which has long been known to inhibit extensor posture of extensor muscles (see Bremer, 1922 and 1935), is capable of inhibiting the vasomotor action.

The results were distinctly positive. They have shown that: (i) weak faradic stimulation of the paleocerebellum (vermian part of the anterior lobe) strongly inhibits the vasopressor reflexes caused by electrical stimulation of a sensory nerve (superior laryngeal, sciatic); (ii) similar paleocerebellar faradization inhibits vasodilator reflexes evoked by electrical stimulation of the central end of the vago-depressor nerve (after double vagotomy; (iii) spontaneous vasomotor waves are equally inhibited; (iv) paleocerebellar stimulation has a depressor action on blood pressure, when the latter is spontaneously raised by a central mechanism (strong bulbar vasomotor tonus), the action failing if blood pressure is low, or raised artificially by a peripheral mechanism (injection of ephedrine); (v) inhibitory action is still present in curarized animals; moreover the depressor action mentioned above in (iv) is sometimes present with stimulation so weak that it is without action on extensor posture paleocerebellar inhibition is thus independent of the classic inhibiting action on postural extensor tonus; (vi) superficial cocaineization of the excitable cortex suppresses the inhibitory action; the latter thus has a corticocerebellar origin.

We conclude from our experiments that there is a direct inhibitory action of the paleocerebellar cortex on the central vasomotor center and that, in consequence, it must be admitted that the cerebellar function affects, not only the somatic nervous activity, but also the autonomic nervous system. It is a well known fact that in the brain stem are the centers of a highly developed reflex vegetative activity, which constantly control the respiration and circulation. In this respect the carotid sinus reflexes play an essential part. It was therefore of interest to examine whether these reflex activities are subject to the control of the paleocerebellum. For the literature on the carotid sinus reflexes we refer to the monographs of Heymans, Bouckaert and Dautrebaude (1933) and of Heymans and Cordier (1935).

An action of the cerebellum on the carotid sinus reflexes has not, to our knowledge, been considered until now. It has been stated recently, however, by Mansfeld and Tyukodi (1936) that the cerebellum, and not the brain stem, would be the center of carotid sinus chemical reflexes acting on respiration. Mansfeld and Tyukodi base their statement on experiments involving decerebration and extirpation of the cerebellum; they observed in fact that after the latter operation the carotid sinus chemical reflexes on the respiration disappeared.

The experiments of Mansfeld and Tyukodi were repeated by Stella

(1937), who did not confirm them. Mansfeld and Hamori (1938) on the other hand have recently insisted on the absence of these chemical reflexes after decerebration and decerebellation. But in a demonstration at the general meeting of the physiological society (1939), Stella has again clearly shown the presence of strong respiratory reflexes from the carotid sinus chemo-receptors in a decerebrate and decerebellate, bulbo-protuberantial dog. The positive results obtained by Stella are decisive evidence against the hypothesis of Mansfeld and his collaborators. The negative results of the latter authors can easily be explained if one considers the close relations (topographical and circulatory) between cerebellum and brain stem, the acute and severe character of the operation, the low level of the decerebration.

It may thus be concluded that vegetative, as well as postural (Magnus, 1914), reflexes are still present in the decerebellate animal; the anatomical localization of all these reflex arcs is chiefly bulbo-pontine. The question then arises whether there is an action of the cerebellum on the underlying bulbo-pontine reflexes. With regard to the postural tonus, it is generally admitted that this is so. Our experiments suggest that, at least so far as the paleocerebellum is concerned, the same conclusions are true in regard to two fundamental vegetative activities, *i.e.*, the vasomotor and respiratory carotid-sinus reflexes.

METHODS

The investigations were carried out on precollicular cats. In order to reduce to a minimum the time of occlusion of the common carotid arteries during the decerebration, we used the following technique, suggested to us by Professor Bremer. A thalamic preparation was made under ether anaesthesia, followed immediately by a precollicular decerebration, without removal of the diencephalon. In this way it was possible to prevent injury to the large arteries at the base of the skull, and to reduce considerably the danger of carotid hemorrhage.

Electrical stimulation of the vermic part of the anterior lobe of the cerebellum was bipolar and faradic (induction coil; about 50 shocks per second). The intensity of the stimulation was, in general, that which provoked the classic inhibition of decerebrate rigidity (*i.e.*, immediately above the threshold level for the tongue). Blood pressure was recorded from the femoral artery in the ordinary manner and respiration by means of a Marey capsule joined to the trachea.

EXPERIMENTAL RESULTS

Inhibition of vasomotor carotid-sinus reflexes by faradic stimulation of the paleocerebellar cortex

Occlusion of one or both of the common carotid arteries is followed by a marked increase in blood pressure and respiration (Pagano-Hering's reflexes). The blood pressure increase is chiefly due to a release of the bulbar vasoconstrictor center from the inhibitory impulses arising in the carotid sinus pressoreceptors. The hyperpnea, at least in the decerebrate preparation, is also related to a reflex excitation of the respiratory center from the carotid sinus chemoreceptors (Stella, 1936). In both cases the background of increased central activity is a very favorable condition for demonstrating an inhibitory action of the paleocerebellum on the bulbo-pontine vegetative

centers; in the same way that the background of increased activity, which is the characteristic feature of the bulbo-pontine tonic centres in the decerebrate preparation, is a very good condition for demonstrating the inhibitory action of the paleocerebellum on the postural tonus.

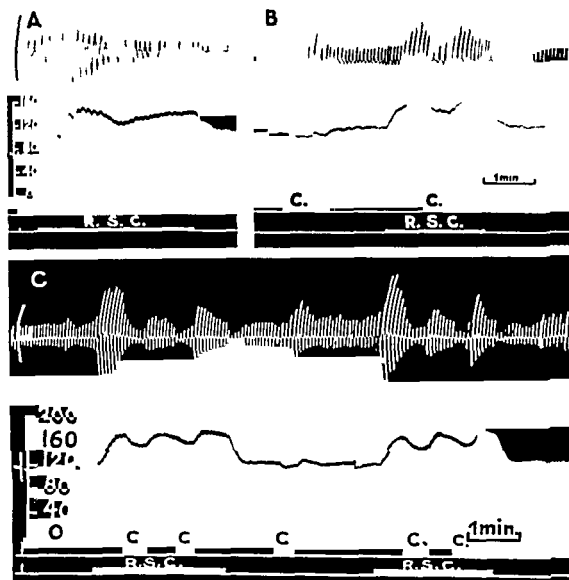


FIG 1 Action of paleocerebellar stimulation on vasomotor and respiratory carotid-sinus reflexes (provoked by the occlusion of the common carotid arteries) In each record from above downwards respiration, arterial blood pressure, c, signal of faradization of anterior lobe, RSC, signal indicating bilateral occlusion of common carotid

A Carotid-sinus reflex of control

B Effects of a paleocerebellar stimulation on normal blood pressure and respiration (left), effects of the same stimulus on carotid-sinus reflex (right) (RSC)

C Another preparation, same type of experiment Note in B and C the small paleocerebellar depressor effects when blood pressure is not raised by carotid occlusion The inhibition of postural extensor contraction was the same in each case

With regard to the action of the paleocerebellum on the vasomotor tonus, we found that weak faradic stimulation of the vermis (lobus anterior) is followed by a marked decrease in blood pressure which had been raised by the occlusion of the common carotid arteries (Fig. 1). The depressor effect may be so strong as to abolish the Pagano-Hering's effect. The inhibitory action is reversible: cessation of the cerebellar stimulation is followed by an

increase in the blood pressure (Fig. 1). It is thus possible, in the course of a few seconds and during the same carotid sinus reflex, to obtain repeatedly the paleocerebellar depressor actions (Fig. 1). We may conclude from these observations that faradic stimulation of the paleocerebellum can counteract, at least in so far as the blood pressure is concerned, the carotid sinus (Pagano-Hering) reflexes.

This antagonism between carotid sinus reflexes and paleocerebellar stimulation is not satisfactory evidence of a central inhibitory effect of the latter. Decrease in blood pressure may result from many different mechanisms. Two observations however strongly support the hypothesis of a paleocerebellar inhibition of the bulbar vasoconstrictor tonus: namely, faradic stimulation of the paleocerebellum has no depressor effect, or only a very feeble one (a) when the blood pressure is low or normal, *i.e.*, not raised by the occlusion of the common carotid arteries (Fig. 1B, 4, 5), and (b) when a low blood pressure is increased by injections of drugs (ephedrine) acting chiefly on the peripheral vasoconstrictor tonus. We may thus conclude that, in order to produce a paleocerebellar depressor effect, the blood pressure must be raised by a central mechanism, *i.e.*, it is necessary to produce a background of excitation in the bulbar vasoconstrictor center. These experimental facts are actually inconsistent with the hypothesis of a pure peripheral antagonism between paleocerebellar stimulation and carotid sinus reflexes, the former decreasing and the latter increasing blood pressure.

The inhibitory action of the carotid sinus circulatory reflexes, produced by faradic stimulation of the paleocerebellar cortex, is not necessarily correlated with the well known inhibition of the decerebrate rigidity produced by the same stimulation. As a matter of fact the vegetative action is still present in curarized animals (Fig. 2), after complete abolition of the peripheral paleocerebellar somatic effects. The vegetative inhibition is thus an independent phenomenon and not merely a consequence (by mechanical means or reflex action) of the inhibition of the postural extensor tonus.

Double cervical vagotomy does not abolish or decrease the inhibition; nor is the paleocerebellar depressor effect characterized (when the vagal innervation is still present) by any important changes in the heart beat frequency. The inhibition thus chiefly affects the vasomotor centers.

Local cocainization (cocaine chlorhydrate 2-5 per cent) abolishes the inhibitory effect on vasomotor tonus, as it does the inhibition of postural tonus. The paleocerebellar faradization effects are thus primarily due to the activity of the faradised cortex. Strengthening the intensity of the stimulation is however followed by reappearance of the inhibitory effects previously abolished by local cocainization; the phenomenon is probably due to spread of physical stimuli to the deep cortical convolutions and to the cerebellar nuclei. It must be remembered here that so far as postural tonus is concerned the activity of the cerebellar nuclei seems to be very similar to that of the cerebellar cortex (Miller and Laughton, 1928; Magoun, Hare and Ranson, 1935). We may conclude from these experiments that the paleocerebellar cor-

tex clearly inhibits the central vasomotor tonus. It is now of interest to examine the main similarities and differences between the well known somatic action of the paleocerebellum on postural tonus and the vegetative effects on vasomotor centers.

There is considerable parallelism between the two paleocerebellar inhibitions: (i) the effects are constantly produced by weak faradizations of almost the same intensity and duration and applied to the same areas of the cerebellar cortex; (ii) in both cases the inhibitions are slow to appear, they

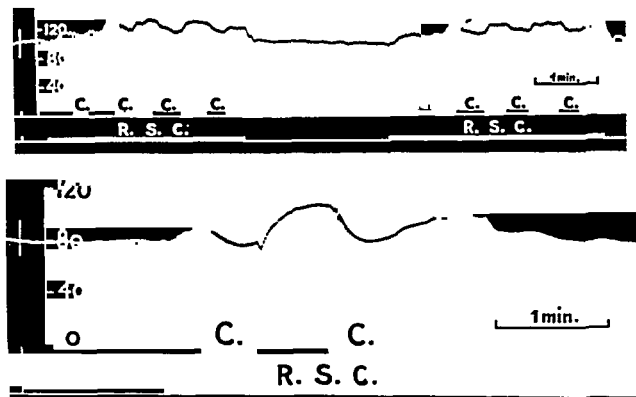


FIG. 2 (Above). Action of paleocerebellar stimulations on vasomotor carotid-sinus reflexes in a curarized animal. Same type of experiment as in Fig. 1, but during a curare paralysis (intravenous injection; 1 mg/kg). Owing to the initial small carotid-sinus reflex, the first paleocerebellar stimulation of every series has no depressor effect; paleocerebellar rebound increases blood pressure.

FIG. 3 (Below). Action of paleocerebellar stimulations on a feeble vasomotor carotid-sinus reflex. Same type of experiment as that shown in Fig. 1. Small depressor effect at the first paleocerebellar stimulation. The rebound increases the blood pressure level; a new paleocerebellar stimulation is followed then by a more marked inhibitory action.

persist during the whole time of stimulation and are followed by marked rebounds; (iii) if the background of activity to be inhibited is small or absent, there is little or no apparent paleocerebellar effect during the stimulation, but the rebound is extremely strong and persistent; a new stimulation strongly inhibits the rebound and is followed by another (sometimes still stronger) rebound (see Bremer, 1922; Bremer and Ley, 1927, Moruzzi, 1936 for the postural tonus; see Fig. 3 for vasomotor tonus).

The main difference between the somatic and the vegetative activities of the paleocerebellar cortex is that the intensity of the paleocerebellar influence seems to be much greater for postural contraction of skeletal muscle

than for vasomotor tonus. As a matter of fact it is always possible to produce a complete paleocerebellar abolition of the postural extensor tonus; so far as the somatic control is concerned, the faradic stimulation of the vermis ceases to inhibit only when there is no more background of central activity to inhibit. Quite different is the paleocerebellar behavior towards vasomotor tonus. There is no depressor action (or only a feeble one) on the normal blood pressure. Faradic stimulation of the excitable vermian cortex may however (as we have seen) abolish the pressor effect of the Pagano-Hering reflex; but in this case too the blood pressure does not fall, under the action of the paleocerebellar stimulation, definitely below its normal level. In other words paleocerebellar stimulation ceases to inhibit when there is still considerable central (spinal and bulbar) vasoconstrictor tonus.

Inhibition of respiration and of respiratory carotid sinus reflexes by faradic stimulation of paleocerebellar cortex

Faradic stimulation of the paleocerebellar cortex has a constant and definite inhibitory effect on the respiration of the decerebrate cat (Fig. 1b). Both the frequency and the amplitude of the respiratory movements are decreased; the inhibition is sometimes followed, at the end of the stimulation, by a powerful rebound which chiefly affects the depth of the inspiration.

The inhibitory effects are much more evident if the respiration has been increased by a carotid sinus reflex. We have investigated the action of a paleocerebellar stimulation on the hyperpnea produced by the occlusion of the common carotid arteries or by intracarotid injections of KCN. Both these hyperpneas are reflex in nature (Pagano, 1900). The first is simply the respiratory aspect of the Pagano-Hering's reflex; the second is due to the excitation by KCN of the carotid body chemoreceptors (Heymans, Bouckaert and Dautrebande, 1931; Heymans and Bouckaert, 1932; Samaan and Stella (1935)).

The general features of the inhibition are the same as those already described for normal respiration. During the persistent hyperpneas produced by occlusion of the common carotid arteries (Fig. 1) or by intracarotid injections of large doses (0.3–0.7 mg) of potassium cyanide (Fig. 4B, 5), it is possible to obtain the inhibitory effects repeatedly and constantly. The respiratory reflexes of short duration produced by intracarotid injections of small doses (0.1 mg) of KCN, are on the other hand much less strong when the injection is made during a faradic stimulation of the paleocerebellum (Fig. 4A).

The paleocerebellar inhibition of respiration is due, like the inhibition of postural or vasomotor tonus, to the activity of the cerebellar cortex which is faradized; in fact local cocainization abolishes the inhibition.

On the other hand the effects on respiration are quite independent of the well known inhibition of decerebrate rigidity. As a matter of fact the former is still present when the latter is completely absent owing to the abolition of postural tonus produced by small doses of curare ("atonie curarique" of

Bremer, Titeca and van der Meiren, 1927). These experiments, like those on vasomotor effects in curarized animals, do not reject the possibility that in normal animals the powerful inhibitions and rebounds in the somatic activity may have some secondary effects on blood pressure or on respiration.* It

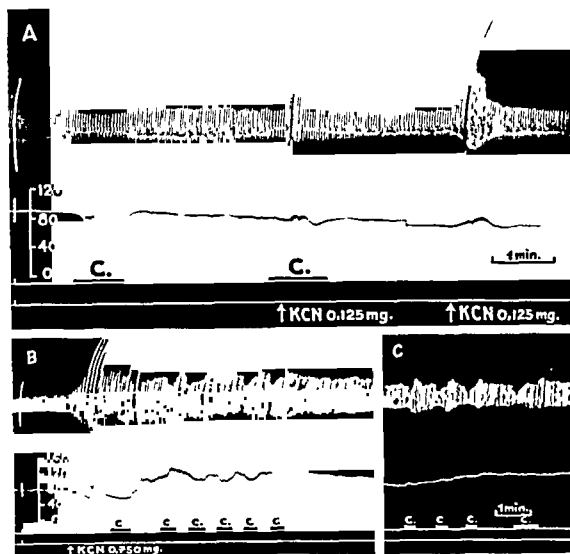


FIG. 4. Action of paleocerebellar stimulations on chemical respiratory carotid-sinus reflexes. Precollicular cat. Reflex activity provoked by intracarotid injections of potassium cyanide (arrow).

A. From left to right: action of a paleocerebellar stimulation (C) on normal respiration; inhibitory action of the same stimulation on a chemical respiratory reflex; respiratory reflex of control.

B and C (another preparation). Action of two paleocerebellar stimulations on a long respiratory reflex provoked by intracarotid injection of a large dose of potassium cyanide. The inhibition affects chiefly the amplitude of the respiratory movements.

must, however, be emphasized that these secondary actions, if present, are not the essential features of the inhibitory effects which we have observed. On the other hand the inhibition of respiration is independent of the inhibition of vasomotor tonus. It is, in effect, possible to have the former when the

* Clear examples of the relations between respiration and postural tonus are reported in Beritoff (1916), Olefrenko (1937) for the decerebrate preparation; in Barcroft and Barron (1937-38) for the foetal animal.

latter is absent owing to the low level of the bulbar vasomotor tonus (Fig. 1B, 4A and C, 5); and the reverse is sometimes true.

We may thus conclude that paleocerebellar stimulation is followed by

(at least) three independent inhibitory effects; the well known inhibition of postural extensor tonus and the inhibitions of vasomotor tonus and respiration. The three effects are clearly due to the impulses arising in the paleocerebellar cortex and acting on bulbopontine centers of the postural and vasomotor tonus and of the respiration. It is, however, necessary to emphasize that the expression "independent inhibitory effects" is nothing but a pure translation in words of the experimental fact that it is possible to have one effect in the absence of the others. It does not mean that in paleocerebellar cortex some units (Purkinje cells) are specialized in the control of somatic functions, others in the control of the vegetative ones; nor that there are no central interrelations between all these activities. The contrary is probably true. It is only necessary to recall here (a) that all the inhibited centers have a bulbopontine localization

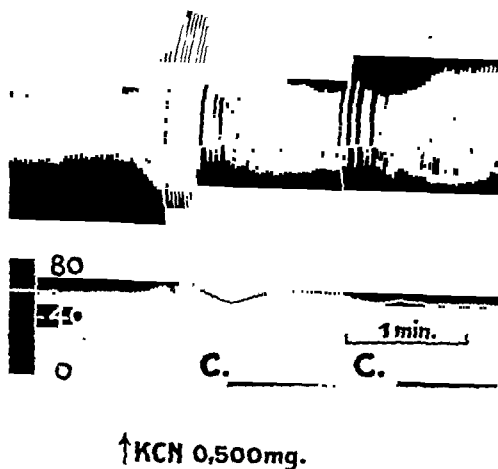


FIG. 5. Action of paleocerebellar stimulation on chemical respiratory carotid-sinus reflexes (produced by intracarotid injection of potassium cyanide). Signals as in Fig. 4. Paleocerebellar inhibition affects chiefly the frequency of respiration; blood pressure is low and therefore almost unaffected by the paleocerebellar stimulation.

tion and (b) that the existence of close functional (purely central) interrelations between these centres is highly probable (see Adrian, Bronk and Phillips, 1932, for the relationships between vasomotor and respiratory centers; Barcroft and Barron, 1937-38, for relations between postural tonus and respiration).

DISCUSSION

The main result of these experiments is the demonstration of a cerebellar action on two fundamental functions of the vegetative life, namely circulation and respiration. Consequently the classic doctrine, according to which the cerebellum is purely a somatic center, is apparently too narrow. The vegetative function of the cerebellum must thus be taken into serious consideration. It should be emphasized, however, that the present experiments only concern the function of the "spinal story" of the paleocerebellum in the decerebrate preparation, i.e., the activity of a comparatively small

part of cerebellar cortex in the simplest possible condition. Whether the present results are to be extended beyond these limits (neocerebellar relations between cerebellum and higher vegetative centers), is still an open question, which will possibly be settled by further investigation; it will not be dealt with here.

We know at present four different types of nervous activity which may be inhibited by a paleocerebellar stimulation. Of these two are somatic, two vegetative in nature. They are: (i) somatic postural contraction, *i.e.*, the inhibition of the decerebrate rigidity which is best known and still the most striking effect of paleocerebellar stimulation (see Bremer, 1935); (ii) effects on spinal reflexes (Bremer, 1922; Denny-Brown, Eccles and Liddell, 1929); this inhibition is still present after elective abolition by curare of the tonic component of the spinal reflexes (Moruzzi, 1935). This indicates that the phasic (kinetic) reflex activity is directly inhibited by the paleocerebellum; (iii) vasomotor tonus and vasomotor reflexes, (iv) respiration and respiratory reflexes. Of these functions three at least (i, ii and iii above) have a bulbopontine localization; thus the control of bulbopontine functions* seems to be the chief aim of the paleocerebellar activity in the decerebrate preparation.

A regulatory control of this kind can only be efficient if it is reflex in nature. The remarkable coincidence between the inhibitory area and the zone of cortical termination of the spino-cerebellar tracts (Bremer, 1922) strongly supports this view. The paleocerebellum may accordingly (and roughly) be described as a system of reflex arcs "in derivation" with the underlying bulbopontine (somatic and vegetative) reflexes. The problem we have to deal with is finally that of the relationships between the bulbopontine and the superimposed paleocerebellar reflexes.

It is of course impossible to give an adequate explanation of the mechanism of these correlations. But two points at least should be emphasized. First, the inhibitory function of the paleocerebellum is not necessarily continuous or "tonic" for all the bulbopontine activities. So far as the regulation of blood pressure is concerned it is quite possible, for example, that the highly developed bulbopontine mechanisms (namely carotid sinus and cardioaortic reflexes) perform the largest part of the work. On the other hand *inhibition* is not the only possible function of the paleocerebellum. The observations of augmentatory paleocerebellar actions on extensor muscles (Denny-Brown, Eccles and Liddell, 1929), chiefly when their postural tonus is absent (Moruzzi, 1936), as well as the paleocerebellar inhibition of vasodilator reflexes (Moruzzi, 1938), might possibly indicate that the aims of paleocerebellar activity should be described as a control more than a simple unidirectional inhibition of somatic and autonomic bulbopontine reflexes.

* That does not imply that all the bulbopontine reflexes are under a paleocerebellar control. Faradic stimulation of the anterior lobe does not inhibit the trismus (Bremer, 1935) or the deglutition reflex (personal observations) of the decerebrate preparation.

It is consequently probable that, in the last instance, the bulbopontine centers impose on the paleocerebellum the nature and intensity of the effort which must be accomplished, both in the somatic and in the autonomic sphere.

SUMMARY

The author's previous experiments have shown that weak faradic stimulation of the vermian cortex of the anterior cerebellum causes strong inhibition, not only (as is well known) of decerebrate rigidity, but also of vasopressor and vasodilator reflexes and of the spontaneous vasomotor waves. The present paper concerns the action of the same cerebellar stimulation on vasomotor and respiratory carotid-sinus reflexes. The following effects have been observed:

1. Inhibition of carotid-sinus vasopressor reflexes brought about by the occlusion of the common carotid arteries. The depressor action on normal blood pressure is less evident or absent.

2. Inhibition of carotid-sinus respiratory reflexes produced by a carotid occlusion as above. There is also less evident inhibitory action on normal respiration.

3. Inhibition of the carotid-sinus reflexes, chiefly respiratory in nature provoked by an intracarotid injection of potassium cyanide.

Experiments of local cocainization, total or partial curarization and occasional observations show that the vegetative effects observed are (like the somatic ones) due to a central inhibition of bulbopontine centers by the paleocerebellar cortex. We must, therefore, admit the existence of a cerebellar control *not only in the somatic but also in the vegetative sphere. The mechanism and the functional significance of the vegetative action of the paleocerebellum are discussed.*

I am deeply indebted to Professor Frédéric Bremer, in whose laboratory this work was done, for his stimulating suggestions and criticisms. I wish to acknowledge the technical assistance of Mr. L. Chantraine.

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BRAIN POTENTIAL CHANGES IN MAN DURING CYCLOPROPANE ANESTHESIA

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IN AN earlier paper (Rubin and Freeman, 1938) it was reported that the intravenous injection of sodium cyanide resulted in the appearance of rhythmic 4 to 6 per sec. waves in the electroencephalograms of a narcoleptic individual and a stuporous catatonic patient. We suggested that this atypical cyanide response was due to a state of functional depression of cortical neurons. To elucidate the mechanisms underlying the production of slow potential changes, we have investigated the influence of cyclopropane anesthesia on brain wave frequencies. Since no systematic study of human brain potential changes in relation to anesthesia have been reported, this study has filled the gap in our knowledge. In addition, it provided the basis for interpretation of alterations in the electroencephalogram resulting from the injection of sodium cyanide at various levels of anesthesia, which will be reported in detail elsewhere.

METHODS

The patients were given a mixture of cyclopropane (trimethylene)* and oxygen to breathe. The induction period with this anesthetic is very short, the average time to reach surgical anesthesia being about 5 min. A total of 36 experiments were performed on 12 subjects, of whom 9 were schizophrenic patients and 3 were non-psychotic individuals. Since no differences were apparent in the data from the 2 groups, no distinction will be made in reporting the results of the experiments. The subjects had no breakfast and were given 1/100 grain of atropine subcutaneously about 15 minutes before anesthetization.

Brain potentials were recorded during each anesthesia period from monopolar disc-electrodes (solder) fixed to the scalp by a drop of collodion. The reference (earthed) lead was placed on the mastoid processes. Two independent, well-matched Grass amplifiers and ink-writing undulators were employed to record the electroencephalograms.

RESULTS

Anesthesia. During the course of cyclopropane anesthesia there are two distinct alterations of the electrical activity of the brain. After a minute or two, the alpha waves decrease in frequency by about 20 per cent and increase in amplitude by 100 per cent or more. (These 7 to 8 per sec. waves are abolished when the subject opens his eyes.) Occasional alpha waves of the original frequency are seen, and slower, random waves appear (Fig. 1, II). It is during the latter portion of this phase that the lid reflexes disappear. The same potential changes may be seen even in those individuals with no alpha activity in the waking state.

The second step is marked by a further increase in amplitude and by the

* We are grateful to E. R. Squibb and Sons for their generosity in supplying us with the cyclopropane.

appearance of rather regular 3 per sec. waves (Fig. 1, III). Again, as in the previous stage, occasional alpha waves of pre-anesthesia frequency are seen. The patient is now in surgical anesthesia, having breathed the anesthetic for 3 to 10 minutes in all. The only exception to this description is found in the case of the occipital lobes, where no regular frequency occurs, even in deepest anesthesia. We shall have more to say about this later.

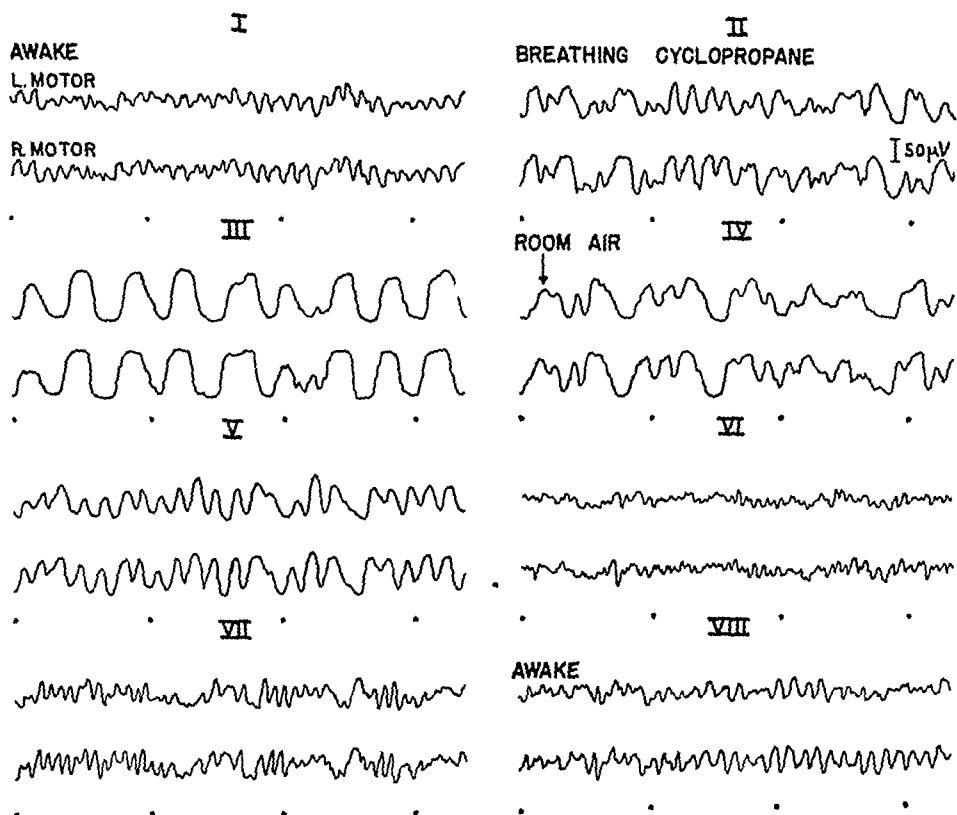


FIG. 1. Simultaneous records from the left and right motor regions, illustrating the changes in frequency during cyclopropane anesthesia and recovery. I, awake; II, breathing cyclopropane for 2 min.; III, 1 min. later; IV, immediately on substitution of room air for cyclopropane; V, 3 sec. later; VI, 2 min.; VII, 5 min.; VIII, completely recovered from the anesthesia. Amplification constant throughout. See text for further description.

Recovery from anesthesia. If the subject has not been kept in deep anesthesia for a great length of time (i.e., more than 3 to 5 minutes), the early stages of recovery are extremely rapid. Immediately upon substituting room air for the cyclopropane mixture, irregular activity of lower voltage with mixed frequency appears (Fig. 1, IV). Isolated waves with durations of $1/30$ to $\frac{1}{2}$ sec. are found in this stage.

On the other hand, when the patient is allowed to breathe room air after he has been in surgical anesthesia appreciably longer than 5 minutes, there is

a further slowing of frequency and the pattern is more irregular than that in deep anesthesia. This is presumably due to the increased absorption of accumulated cyclopropane which is occasioned by the change from shallow to deep respiration on breathing room air.

In the next stage, which follows the preceding one by a few seconds, there is an almost continuous 7 to 8 per sec. rhythm (Fig. 1, V). In the course of the next few minutes the amplitude drops progressively and the 7 to 8 per sec. waves disappear.

The third step in the recovery is marked by low-voltage (10μ V) potential changes of higher frequencies. The predominant frequency is 18 to 20 per sec., with occasional 10 per sec. and more numerous 12 to 14 per sec. waves interspersed (Fig. 1, VI). This stage may not occur in some subjects, in which case there is a very short period of relatively flat baseline with random waves of no fixed frequency.

In the last stage before complete recovery there is an increase in amplitude and a decrease in frequency, 12 to 14 per sec. waves predominating (Fig. 1, VII). In some subjects this rhythm may occur almost continuously for several minutes; in others it may appear in short bursts with relatively little activity in the intervals. In the next 5 or 10 minutes there is a gradual increase in the number of alpha waves, accompanied by a decrease in the number of 12 to 14 per sec. waves.

The subject may be easily aroused during this stage, as evidenced by his response to simple questions and by the sharp increase in the number of alpha waves appearing in the electroencephalogram. However, he soon slips back into a drowsy state, and if undisturbed will remain in this state for many minutes. Arousing the subject several times appreciably shortens the duration of this stage, so that he is fully conscious and exhibits the pattern of electrical activity characteristic for him in the waking state minutes sooner than would have been the case had he been left undisturbed. This is in direct contrast to the 14 per sec. stage in normal sleep, where the subject is in deep sleep and can be aroused only with great difficulty (Loomis *et al.*, 1937; Davis *et al.*, 1938).

Analysis of frequency changes. In describing the frequency changes in the previous section, predominating rhythms were employed as criteria. It is probably more significant to consider the shift which occurs during anesthesia in the frequency spectrum as a whole. This has been done in the following manner.

Eight consecutive seconds of record typical of each stage were selected and the duration of every potential change was measured with a millimeter rule. The speed of the recording tape was 30 mm per sec., so that duration in mm could be readily translated into fractions of seconds, and finally into number of waves per sec. Since this method is not sufficiently sensitive in detecting very small differences in wave duration, the data were treated as groups of frequencies. For example, a group with a range from 0.5 to 5 per sec. corresponds to delta activity, 8 to 10 per sec., in this individual, is the range of alpha-wave frequency, 10.5 to 15 per sec. includes the 12 to 14 per sec. waves characteristic of one of the stages of recovery from anesthesia, etc.

The number of waves (frequency of occurrence) of the various frequency groups in the 8-sec. sample were then plotted as a function of time (Fig. 2).

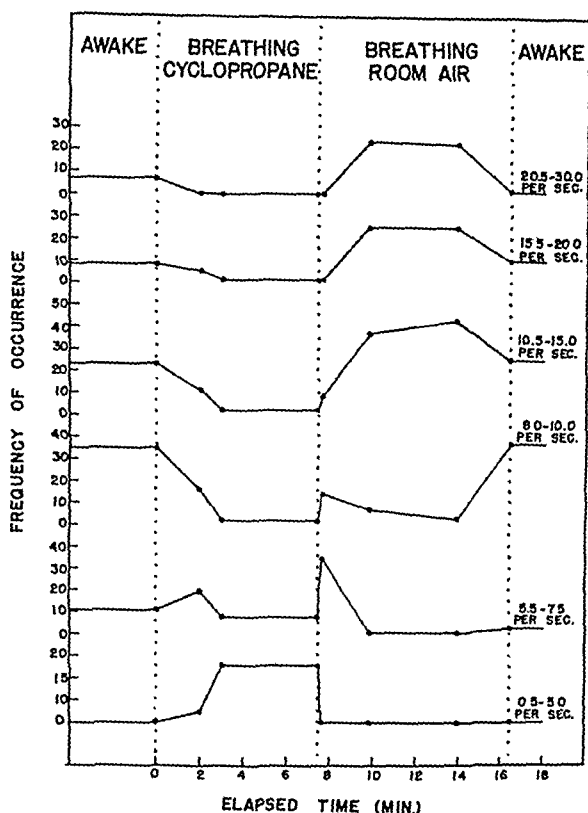


FIG. 2. A quantitative description of the number of waves of various frequencies observed in the several stages of a typical cyclopropane anesthesia.

possible (see Fig. 1). On the other hand, simultaneous tracings from the various architectonic regions of the cerebral cortex reveal marked differences which may be listed under 4 categories.

(i) *Time of onset of slow activity.* Slow potential changes appear earliest in the anesthesia in the frontal region. The motor and premotor regions are next, and the occipital and parietal regions the last to show 0.5 to 7.5 per sec. activity. The parietal is the most variable in this respect, sometimes being similar to the motor region and sometimes similar to the occiput.

Actually it is difficult to make a reliable generalization as to which regions manifest slow activity first. When differences in the time of onset of slow activity do occur, they are seldom greater than a few seconds. This may be due to the rapid rate of induction by cyclopropane, with the result that the separate phases of electrical activity associated with anesthesia are crowded together and not easily recognizable as discrete stages. Differences

It is interesting that the alpha rhythm is commonly present to some degree in its original frequency throughout the several stages of anesthesia and recovery from it.* In this particular case (Fig. 2) an exception to this statement is found in the stage of deepest anesthesia. It may be seen in Fig. 2 that the frequency changes in the approach to anesthesia are not mirrored in recovery from anesthesia. This has also been observed in normal sleep (Loomis, Harvey and Hobart, 1937; Blake, Gerard and Kleitman, 1939).

Architectonic distribution of frequency changes

A. The electrical patterns from corresponding regions of the two sides of the head during anesthesia and subsequent recovery from it are nearly superimposable

* Alpha waves have also been reported during normal sleep (Blake and Gerard, 1937).

in time of onset of slow waves may be more clearly seen in sleep, where the diminution of consciousness is more gradual. For example, one of our patients became drowsy before the anesthetic was administered. In Fig. 3 are shown strips of record taken while the subject was drowsy and after he was awakened. A comparison of the tracings shows that the electrical activity of the parietal lobe was unaltered by the drowsiness, whereas the premotor region exhibited a regular 4 per sec. rhythm of increased voltage with alpha and 12 to 14 per sec. waves superimposed.

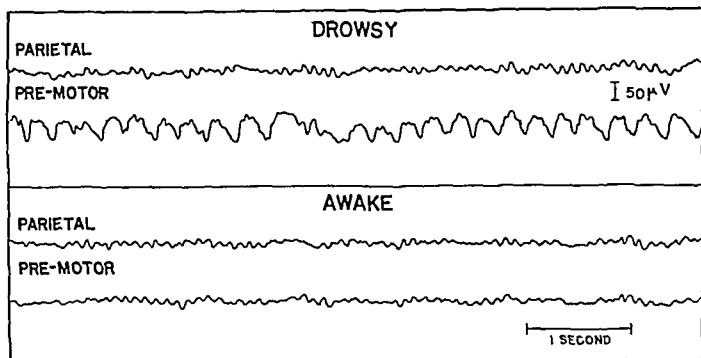


FIG. 3. Simultaneous records from the right parietal and premotor regions of a subject when drowsy and when subsequently awakened, illustrating the more rapid onset of slow potential changes in the premotor region and the independence of the 2 regions. Amplification the same in both records.

(ii) *Recovery rate.* Usually the motor and premotor regions are the first to recover from anesthesia. These two areas are then followed in recovery by the occipital and parietal regions. The frontal lobes are the last to show the waking potential pattern. The difference in recovery time may be as great as several minutes in some instances.

(iii) *Amplitude.* Amplitude of the slow waves during anesthesia is lowest in the occiput and increases progressively as one records anteriorly to the premotor region. Slow activity in the frontal lobes is as great as, and sometimes greater than, that in the motor and premotor regions.

(iv) *Regularity.* The most regular, slow potential changes are observed in the frontal lobes. This is less true of the areas posterior to the frontal region. Regular, slow rhythms are rarely seen in the occipital lobes, even in deepest anesthesia, although it is true that occipital delta waves may be of longer duration than those occurring elsewhere in the cortex.

Some of the preceding points are illustrated in Fig. 4. In addition, it illustrates another interesting point. During the latter part of the anesthesia,

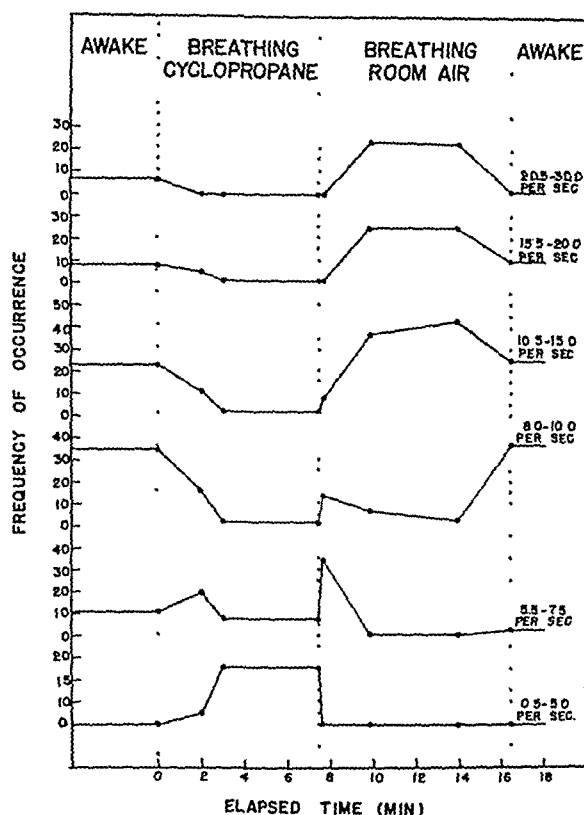


FIG. 2 A quantitative description of the number of waves of various frequencies observed in the several stages of a typical cyclopropane anesthesia.

posable (see Fig. 1). On the other hand, simultaneous tracings from the various architectonic regions of the cerebral cortex reveal marked differences which may be listed under 4 categories.

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Architectonic distribution of frequency changes

A. The electrical patterns from corresponding regions of the two sides of the head during anesthesia and subsequent recovery from it are nearly superimposable

* Alpha waves have also been reported during normal sleep (Blake and Gerard, 1937).

rhythm is low in amplitude (very seldom above $20 \mu\text{V}$) and appears in short, random bursts. The 12 to 14 per sec. stage is seen almost always in the motor and premotor regions, where it sometimes may occur as a more or less continuous discharge lasting for as long as a minute or more with an amplitude of $75 \mu\text{V}$. This stage is not as marked in the frontal lobes.

C. Synchronization. With the exception of the occipital lobes, neighboring regions may exhibit such similar activity, especially in deep anesthesia, that their records are virtually superimposable. However, potential changes under electrodes as much as 10 or 12 cm. apart may also occasionally become synchronized. In the waking state the tracings from the premotor region and forehead are quite different (Fig. 5a). In deep anesthesia they are still clearly dissimilar (Fig. 5b). For brief intervals, however, the two regions may show good synchrony (Fig. 5c), then reverting to a state of pronounced asynchrony (Fig. 5d). This alternation may be repeated several times during the anesthesia, but never, in our experience, are the periods of synchrony of more than a few seconds in duration.

In general, the cortex seems to act in 3 units as far as similarity in potential changes is concerned. During anesthesia and recovery from it, the activity is more or less the same throughout the frontal region; the motor and premotor regions form another unit; and, finally, the parietal and occipital areas form a third unit. There are exceptions to this, in that the parietal lobes may, in some instances, show the same electrical activity as the motor and premotor regions, and, less frequently, the frontal and premotor regions may beat in unison. These observations are in harmony with the view that the various cortical regions in man are relatively independent of each other in the production of their electrical activity (Jasper and Andrews, 1938; Rubin, 1938). In their study of the distribution of potential changes during normal sleep, Loomis, Harvey and Hobart (1938) report that right and left halves of the head show similar patterns while front, top

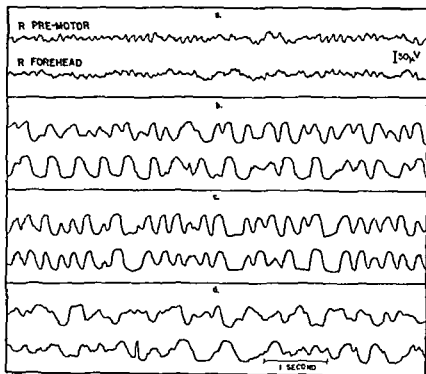


FIG. 5. Simultaneous records from premotor region and forehead, illustrating asynchrony in *a* (awake) and *b* (anesthesia); synchrony in *c*; and reversion to asynchrony in *d*. *b*, *c* and *d* taken during the same anesthesia. Amplification constant throughout.

and back may be quite different. The distribution during cyclopropane anesthesia, therefore, is essentially the same as that in normal sleep.

Variability. The course of anesthesia is not a smooth one, as judged by the observed potential changes. Frequent fluctuations between successive stages are seen superimposed upon a more gradual, unidirectional trend. This is especially true in the later stages of recovery from anesthesia.

There is a remarkable constancy of the potential patterns which a given individual exhibits in repeated anesthetics on the same day or from day to day. Although there is less differentiation between individuals in anesthesia than in the waking state, there are certain distinguishing characteristics. For example, some subjects may be brought to surgical anesthesia in 3 minutes or less, whereas it takes 10 minutes or longer for others. During surgical anesthesia the frequency may go as low as 0.5 to 1 per sec. in some individuals, but in others the same region may never show a rhythm slower than 4 to 6 per sec. The most common frequency we observed in deep anesthesia was 3 per sec. By utilizing a variety of criteria of this sort, one can distinguish between individuals with a fair degree of accuracy.

Influence of CO₂. In the majority of the experiments the CO₂ exhaled by the patient was absorbed by soda lime. However, in some instances it was necessary to allow the patient to rebreathe his own CO₂ at an early point in the anesthesia to prevent respiratory failure. This always hastened the onset of deep anesthesia and made the slow rhythms characteristic of this stage much more regular than usual.

DISCUSSION

If the changes in frequency observed during cyclopropane anesthesia and recovery from it were due solely to a decrease in metabolism of neurons, we should expect them to be gradual and unidirectional (*i.e.*, either progressively increasing or decreasing in frequency as the case may be). Brain metabolism does decrease during anesthesia (Serota and Gerard, 1938). However, the decreases in frequency observed in the electrograms during anesthesia are quite abrupt. The series of frequency changes during recovery from anesthesia (*i.e.*, from 3 per sec.→mixed 3 to 30 per sec.→7 to 8 per sec.→18 to 20 per sec.→12 to 14 per sec.→10 per sec.) is even more difficult to explain on a metabolic basis. Undoubtedly other factors must be involved. For instance, cell permeability is decreased during anesthesia (Spiegel and Spiegel-Adolf, 1936). This, in turn, must influence the ionic relationship between the cells and their environment, which may be reflected in frequency and amplitude changes (Libet and Gerard, 1939). In addition, a blocking of afferent impulses may occur during anesthesia (Beecher, McDonough and Forbes, 1938). Such a blocking is effective in altering the frequency of cortical potentials (Bremer, 1935). All the preceding factors probably contribute in bringing cortical neurons to various levels of excitation at which synchronization can occur. Adrian (1937) has presented evidence that fixed rhythms can occur only when synchrony is good,

and that synchronization in a given group of neurons is a function of the frequency of discharge of individual neurons at a given moment. The present data seem to show several such excitation levels with rather sharply defined thresholds.

Although such a mechanism as just described would hold whether one assumed that different neurons or the same neurons were responsible for various frequencies, the evidence is definitely in favor of the latter view. Adrian's (1937) data indicate that the same neurons can discharge at many frequencies. Libet and Gerard (1938) have demonstrated that the same neurons in the isolated frog brain are capable of "beating" at frequencies from 1 to 50 per sec. The appearance of many distinct frequencies over a period of a few minutes after prolonged insomnia (Blake, Gerard, and Kleitman, 1939) and our present observations of abrupt frequency changes during cyclopropane anesthesia are also in accord with this point of view.

SUMMARY

1. There are 6 discrete changes in the frequency of brain potentials in man during cyclopropane anesthesia and recovery from it. The frequency changes in the approach to anesthesia are not mirrored in the recovery from anesthesia.

2. Potential patterns from corresponding regions of the head during anesthesia and recovery are almost identical. However, the front, top and back of the head form 3 relatively independent units of activity. Differences in time of onset of slow activity, rate of recovery, amplitude and regularity of potentials in the 3 units are described.

3. Just as in normal sleep, a well defined 12 to 14 per sec. rhythm is found most predominantly on top, less so in front and least commonly in the back of the head. This rhythm differs from that in normal sleep in that it appears late in the recovery from the anesthetic.

4. Although there is not as great individuality of potential patterns during anesthesia as in the waking state, it is nevertheless possible to differentiate between anesthetized individuals on the basis of their electroencephalograms.

5. CO₂ hastens the onset of deep anesthesia and makes slow rhythms more regular.

6. Factors which may contribute to the frequency changes seen in cyclopropane anesthesia are discussed.

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CONDITIONED VESTIBULAR REACTIONS

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THE EXPERIMENTS described here arose from interest in the possibility of using vestibular responses as a basis on which to form conditioned reflexes, and in the pathways involved in their establishment. This problem grew out of recent work of this laboratory in: (i) describing the type of physiological reactions which can be conditioned and (ii) delimiting the elements of the segmental and suprasegmental structures essential in the formation of the conditioned response (Gantt 1937a and b; Brogden and Gantt 1937).

In spite of numerous physiological and anatomical studies on this question our knowledge of the representation of the vestibular apparatus in the cortex is still meager. Furthermore certain mental diseases show changes in the irritability of the vestibular apparatus which seem to be related to the irritability of the cortex in general (Aubry and Baruk 1929; Joo and Meduna 1935; Lowenbach 1936). Here also nothing is known of the pathways and centers having to do with the disturbance.

METHODS

Of the three methods of producing vestibular reactions: (i) acceleration, (ii) caloric irritation, (iii) galvanic stimulation, we have used the third because of its ease of application in animals. We were fully aware that this stimulus was the most "inadequate" of the three and had some other disadvantages. The galvanic reactions are produced not by direct stimulation of the semicircular canals but by stimulation of the vestibular ganglion (Dohleman 1926, 1929). The subjective feelings, as known from experiments in man, begin a few seconds after the make of the current. As we used the slightest movement of the head as an indicator of vestibular reaction, and as we know from the work of Bourguignon (1927) and others that such movements follow almost immediately upon the stimulation, the delayed appearance of subjective feelings was of no significance for our experiments. The fact that the break of the current constitutes an additional stimulus is another obstacle. We tried first to overcome this difficulty by not breaking the current abruptly, but decreasing it gradually for a longer period of time. However, this did not abolish the stimulating effect. The experiments later demonstrated that in the dogs which were used, the make of the current was most significant and after a short while the animals neglected the break stimulus. Electrodes made out of copper wire thickly covered with gauze and soaked in saline solution were introduced into both external auditory meatuses, held in their place by a bandage, and the animals blindfolded. The electrodes were connected with a 15 volt battery through a potentiometer and currents between 0.5 and 10 millamps applied (unconditioned stimulus). The conditioned stimuli were a bell, a tone, or a buzzer acting for 1 to 3 seconds before the onset of the galvanic shock and continued 1 or 2 seconds after the shock.

RESULTS

The elaboration of conditioned responses was attempted in four dogs. *Vera*, a placid, quiet and phlegmatic female about 12 years old, had been used several years previously for the study of conditioned food responses. *Vespasian*, a male hound about 3 or 4 years old, within the past year had

elaborated conditioned defense reactions to an electric shock. *Vestus* and *Vesal*, had been used similarly to *Vespasian*.

The unconditioned reflex to the galvanic shock consists in an initial jerk of the head toward the side of the positive electrode, a lowering of the head so that the nose frequently touches the floor and elevation of the negative ear to as much as 90 degrees, a change in muscle tonus on the two sides so that the animal resists having his head or his body pushed to the negative side, and sometimes bending of the body to the positive side and going around in circles toward the anode. Most constant of these reactions was the elevation of the ear and the turning of the snout to one side. Occasionally the dog made side-stepping movements or even fell over. Symptoms of nausea, licking out the tongue, and retraction of the lips sometimes appeared. The positive conditioned response was recorded if one or more of the above components of the unconditioned reactions were observed.

The first conditioned reactions were those of a defense nature, mild to violent struggling according to the temperament of the dog, trying to remove with the forefeet the electrodes from the ears or the bandage from the head. As the conditioned responses changed from defense to specific reactions based on the pattern of the unconditioned reflex, the dog began at first to sway from one side to the other. This swaying accompanied a change of muscle tonus on the two sides. Gradually various elements seen in the unconditioned reflexes appeared as components of the conditioned responses.

After the full development of the specific conditioned response, differentiation was tried in two dogs. In order to accomplish differentiation the direction of the current was reversed, so that the movements of an opposite nature were elicited by this reversed current (elevation of the other ear, movement of the head to the opposite side, circular movements counter-clockwise instead of clockwise, increased muscle tonus on the opposite side, etc.). A new conditioned stimulus was used for the establishment of the new conditioned response. After a certain number of trials the new conditioned stimulus produced movements similar to the new unconditioned reflexes. During the early use of the new signal it elicited the same conditioned responses as the first conditioned signal had, as is generally the case until a differentiation is firmly established.

Vera had been used in 1936 for the establishment of conditioned responses both to food and to acid. These were readily formed after 10 to 15 trials. Experiments on the vestibular reflexes began on the 15th of January 1939. The animal was used in the camera where the food reflexes were previously established. After the 10th trial, using the tone as the conditioned signal for the vestibular reflexes, the animal no longer turned to the food box but began to give specific conditioned vestibular reactions. This at first consisted in turning the head slightly to the left (same side as unconditioned vestibular reflex but opposite to former food conditioned reflex). The latent period was short—about $\frac{1}{2}$ to 1 second. This component, swaying and turning the head to the left, continued after the 11th combination with oc-

casional failures. Later an elevation of the right ear and lowering of the head was added, being constant after about the 100th trial. These reactions were regular and constant until the 211th trial on February 21st, 1939. Vera became very quiet and gave practically no other movements than the specific conditioned responses and the unconditioned reflexes to which latter were occasionally added slight whining after the shock. On the 20th of April differentiation was begun. Retention of the conditioned reflex to the tone was perfect for this two month rest period. A buzzer was used as the new conditioned signal for the reversed current. For the first six trials Vera gave the same conditioned responses to the buzzer as she had to the tone, opposite to those of the new unconditioned reflex, but beginning with the 7th repetition of the buzzer she began to give the appropriate new conditioned reflex—movement of the head to the right, turning to the right, with continued lowering of the head. Elevation of the left ear began only on the 37th repetition of the buzzer and continued thereafter infrequently; the elevation of the right ear completely disappeared after the 34th repetition of the buzzer. The head was bent to the right 15 to 60 degrees. An indifferent sound, a bell, was used to test for generalization; there was absolutely no reaction.

Vespasian had been used several months previously in experiments in which a bell was the signal for a shock on the thorax, the conditioned response being raising of the right foot. On February 21st, 1939, vestibular conditioning was started. At this time the muscular tonus was the same on the two sides. When blindfolded and prepared for the experiment he tucked his tail but remained quiescent. A tone of 1000 c./sec. was the conditioned signal, followed in three seconds by the unconditioned stimulus—a current of 2 to 10 mA, the right ear being positive. There was no reaction to the tone when first used. This dog like Vera showed little defense. The unconditioned reflex consisted in rotating the head so that the left ear was uppermost, occasionally falling over, stepping to the right and running around in circles to the right. Later, the head was lowered so that it was pressed flat on the floor, the right ear being often brought in contact with the floor. With the strong current there was occasional whining.

The conditioned reflex was first seen on the 8th repetition of the tone, consisting of a running to the right and a slight rotation of the head with the left ear up. The movements of the conditioned reflex fluctuated from the 8th to the 72nd repetition; during this time the dog went to the left as often as to the right. There was often a slight swaying of the head from side to side. The presence of nausea was suggested by marked salivation, labored breathing, and lowered head, but this was always transient and the dog readily ate as soon as the bandage was removed from the eyes. The conditioned reflex became stable after the 75th trial on the 25th of February, and was often more marked than the unconditioned reflex. As it became more stereotyped the dog would lower the head nearly to the floor or even on the floor with an elevation of the left ear, and sometimes there was a loss of balance and falling during the conditioned reflex. A posture with the head

rotated, the left ear always being uppermost, was often assumed in the intervals. Fewer extraneous movements occurred so that there was no struggling and only the conditioned reflexes were present. A bell introduced after the 149th repetition of the tone produced no response, showing that there was no generalization. After the tone had been given for 179 times, the buzzer was used for differentiation. During the first trial there was a conditioned reflex to the buzzer. For the purpose of differentiation the electrode in the right ear was changed from positive to negative, thus producing an unconditioned reflex of opposite nature—rotation of the head with *right* ear up, turning of the head toward the *left* and going toward the *left*. Occasionally instead of this appropriate unconditioned reflex the former one appeared.

After the fourth repetition of the buzzer a conditioned reflex occurred, but instead of being equivalent to the new unconditioned reflex it was identical with the conditioned reflex to the tone. Differentiation took place in stages. The turning of the animal to the left instead of to the right, first seen on the 8th repetition of the buzzer, was not constant until much later—after the 250th trial. The rotation of the head in the proper direction, right ear uppermost, began to supplant the old movement of left ear up on 38th repetition. For a long time differentiation was imperfect and until the 237th repetition about one half of the time the head would be turned to the right as it had been with the tone. Later the new conditioned reflex became constant. Conditioned turning of the whole body to the left—a component of the new unconditioned reflex—appeared first on the 189th trial of the tone and occurred fairly frequently after the 248th repetition.

Very striking in this dog, and in this dog only, were the *accessory motor phenomena*, which became prominent during the differentiation period. The postures resembled those described by Pavlov as hypnotic and cataleptic but were even more marked. At times the animal became practically non-reactive to many diverse external stimuli as pinching, loud noises, or needle-pricking, but paradoxically he was nearly always reactive to the conditioned stimulus though apparently asleep. A peculiar position was often assumed for long periods. Sometimes the head was lowered to the floor in a praying attitude, sometimes the body was curved into a complete circle so that the snout touched the hip, at other times the dog curled on the floor, breathed slowly and snored. Such an apparent sleep would last for as long as 15 minutes during which the animal was non-reactive except to the conditioned stimulus, to which he would usually give a slight response. Although some of these reactions were first seen on February 1, the sleep and snoring did not become prominent until differentiation was introduced on March 20. During such sleep the respiration rate was 9 per minute and the heart rate 55–65 compared with 85 at the instant of awaking and 110–120 while eating. The dog could be put into various positions, all of them very unnatural for a dog, without resisting. The knee-jerk was very active during sleep. He did not notice food placed under his nose, although he readily ate it as soon as bandage and electrodes were removed. He was non-reactive to pricking with

hypodermic needles in the legs and over the scalp and also touching the penis, which was frequently partially erected. His limbs were more flaccid than rigid. The appearance of this sleep became routine during differentiation, but gradually became less frequent after the 150th repetition of the buzzer. The conditioned reflex during sleep consisted usually in only a slight rotation of the head.

The development of the conditioned vestibular reflex occurred without difficulty in the two other dogs. Differentiation was not tried in these dogs.

Tested for retention after a five month rest interval in Vespasian and an eight month interval in Vera they both showed perfect retention of the positive vestibular conditioned reflexes to the buzzer. This compares favorably with the retention of food conditioned reflexes, indicating the stability of the vestibular conditioned reflexes.

DISCUSSION

This study establishes the fact that definite and specific vestibular reflexes can be conditioned. The second interest as stated above, concerning the pathways involved, is to be the subject of further investigation. The responses which we conditioned were so similar to the unconditioned vestibular reflexes produced by the galvanic current—often even much more pronounced—that we feel justified in concluding that they were specific vestibular reactions without admixture of defense reflexes. Most of the dogs gave practically no defense reflexes, except for an occasional slight whine, after the first few days of experimentation. The specificity of the conditioned reflex elaborated is further proven by the ability of the animal to differentiate, thereby giving specific movements in the opposite direction to those previously elaborated in the first stage of experimentation. That several elements of the unconditioned reflex were so exactly duplicated in the conditioned reflex is evidence against the argument that the animal might be making conditioned orienting reactions based on the tactile stimulation necessarily accompanying the make of the current.

There are important reasons why the reactions which we conditioned cannot be considered as arising from proprioceptive sensations following the unconditioned movement of the head. First, our reactions occurred very quickly, while proprioceptive conditioned reflexes are formed, if at all, only after hundreds of repetitions. Furthermore, in experiments performed previously in this laboratory, a movement produced by direct stimulation of the motor area of the cortex could not be conditioned.

The method employed to evoke the unconditioned reflex had, as stated in the beginning, the disadvantage of producing with the break of the current a separate complex of vestibular reactions opposite to the reactions to the make of the current. This disadvantage, however, gave further proof of the specificity of the reactions. After a very short period, when the dogs obviously were confused and did not give clear-cut responses to either the make or the break of the current, they learned to relate the tone or the buzzer only

to the make, and the conditioned reflex was then quickly established. Sometimes the dogs, after they had already given the proper conditioned reflex to the conditioned signal and received the reinforcement with the make of the current, would turn their heads to the opposite direction shortly before the current was broken again. In such a manner they showed a conditioning also to the break of the current.

The vestibular reactions, while not clearly autonomic, are closely allied to certain cerebellar reflexes, *e.g.*, those involving "tonus" and equilibration (Clark, 1939). Both vestibular and cerebellar reflexes are generally considered as "involuntary" movements, such as the withdrawal of the paw from a painful stimulus. Also there does not seem to be any essential difference in differentiation and retention of these types of reflexes. Other components of vestibular responses, as nystagmus, nausea, etc., and conditioning to "adequate" stimuli, are the subjects of investigations in progress.

SUMMARY

In four dogs, using a galvanic current between the external auditory meatuses as unconditioned stimulus to produce vestibular reflexes (loss of balance, falling to one side, characteristic head and body movements), these reflexes readily appeared as conditioned responses to an auditory stimulus. Differentiation in these animals was also attained (by reversing the current to produce opposite movements which became conditioned to a new auditory stimulus). Prolonged sleep and peculiar motor phenomena developed in one dog. The conditioned vestibular responses persisted without practise for at least eight months.

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MODIFICATION OF THE CORTICAL FREQUENCY SPECTRUM BY CHANGES IN CO₂, BLOOD SUGAR, AND O₂*

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MANY WORKERS are now agreed that electrical activity of the cortex is greatly modified by changes in blood sugar (1, 2, 3, 4, 5, 6, 7, 8), by changes in blood O₂ (5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18), and by changes in blood CO₂ (5, 12, 13, 14, 15). There is some dispute, however, about the exact nature of these modifications and their possible quantitative relation to any given blood constituent. Data from all previous studies have been based on a visual analysis of the electroencephalogram. Such an analysis is necessarily highly subjective. Changes in frequency which may appear sufficiently obvious to one observer to be easily quantitated by counting and averaging wave lengths, may not appear at all obvious to another. A purely objective method of analysis has been devised recently by A. M. Grass (20). We have in this study used the Grass method of analysis for extending the work of Lennox, Gibbs and Gibbs (5) on the alteration in electrical activity of the cortex with changes in various normal blood borne constituents.

METHODS

Grass method for cortical frequency spectra—The electroencephalogram is taken as a shadowgram on film. Any desired sample of the record is made into a continuous belt and rotated between a light and a photoelectric cell. The resulting fluctuations in potential in the cell are amplified and passed through an exceedingly sharp continuously variable filter, which is connected with a recording galvanometer. By this means, it is possible to record automatically the amount of energy in all frequencies over a wide range. The expression which one obtains is a spectrum, or more accurately, the Fourier transform of the electroencephalogram. It is the most adequate compact expression obtainable for this type of record.

The value of such an expression was appreciated by Dietsch (21) who in 1932 made a harmonic analysis of the electroencephalogram. His figures do not give continuous spectra but represent only 18 points in the spectrum. Although his data in each case were obtained from measurements on only a few waves, each analysis required many hours of careful measurement. Similar mathematical analysis of a few waves was carried out by Rohrer (22) who used 24 ordinates. That these studies are not more significant is due to the inadequacy of the sample used and to the fact that despite much labor, the spectrum obtained is non continuous. With the Grass method of analysis, in five minutes one can obtain a continuous spectrum of the energy in all frequencies from 1 to 60 per sec in a strip of record 30 sec in duration. The electroencephalogram which was analyzed was in all cases, that obtained with a "stigmatic" electrode on the scalp in the left occipital area and "indifferent" electrodes on both ears.

Method of studying blood constituents—The chief source of error in the present study

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does not lie in the recording nor in the analyzing of cortical activity but in the proper quantitation of the blood constituents which alter the activity of the cortex. Lennox and E. L. Gibbs (25), Gibbs, Gibbs and Lennox (26), and Gibbs (5) have shown that respired air, arterial blood, and venous blood from the arm or leg are poor indicators of the CO_2 tension in the brain because the cerebral blood vessels are actively engaged in maintaining the CO_2 tension of the brain relatively constant despite changes in the CO_2 tension of the arterial blood. When the CO_2 in the artery falls, the cerebral blood vessels constrict reducing cerebral blood flow and so conserving CO_2 in the brain, while the limb vessels dilate, allowing CO_2 to be washed out of the limbs. With the CO_2 from the limbs swept into the general circulation, the drop in arterial CO_2 is somewhat checked and the cerebral blood vessels need not constrict so far in order to keep the CO_2 tension of the cerebral blood relatively constant. The constriction of the cerebral vessels with low CO_2 however, becomes less effective as the brain approaches anoxia. To be more specific, when the venous blood returning from the brain is 40 per cent saturated with O_2 instead of 50 to 60 per cent saturated as it is normally, the constrictor effect to low CO_2 begins to fail: When the venous blood from the brain is 28 per cent saturated with O_2 , the point at which consciousness is lost, the constrictor effect to low CO_2 disappears. This failure of the constrictor effect of low CO_2 is in large part responsible for the dilatation which accompanies low O_2 . Added to this, however, there appears to be a direct dilator effect of low O_2 on the cerebral vessels. No change in cerebral blood flow has been detected as a result of varying the blood sugar level.

In order to get close to the nerve cell environment, we have taken blood from high up in the internal jugular vein. It would have been better perhaps if data could have been obtained on the tissue fluid of the brain directly under the "stigmatic" electrode, as has been done by Dusser de Barenne, McCulloch and Nims (19). But what we have lost in directness, is partly made up for by the certainty that the technique we have employed could not possibly have injured normal responsiveness of blood vessels or nerve cells. Blood from the jugular vein at a point just below the jugular bulb is under ordinary conditions in equilibrium with the tissue fluids of the brain. It should reflect fairly accurately all gradual changes in diffusible substances. Sudden changes, however, will not be accurately reflected. In order to avoid error due to lag, all changes in blood constituents were made slowly and samples were taken when it was believed that equilibrium had been established. All CO_2 and O_2 determinations were done by the Van Slyke manometric method, sugar determinations were done by the Folin-Wu method.

Material—All subjects were adult men whose brains were normal. In the studies on high O_2 pressure, however, rabbits were used because the high O_2 pressure necessary to produce marked changes in the cortical frequency spectra are so high that they are difficult to maintain and properly control for an animal as large as man. The cerebral venous blood was not studied in these high O_2 experiments. It seemed reasonable to assume that the increase in O_2 pressure in the arterial blood, which occurs with an increase in atmospheric pressure, is due to an increase in the amount of O_2 in simple solution. Thus, the rise in O_2 pressure in the arterial blood will be a direct function of the partial pressure of O_2 in the atmosphere. Nevertheless it is hazardous to use the O_2 pressure in the arterial blood as an indicator of the O_2 pressure in the brain. The technical difficulty of taking blood samples under such high pressures caused us to proceed without determining the O_2 content of the cerebral venous blood, especially as we were not so much interested in the exact O_2 pressure at which changes in the cortical frequency spectrum occur, as in establishing the fact that certain changes do occur and that they occur at a critically high O_2 pressure.

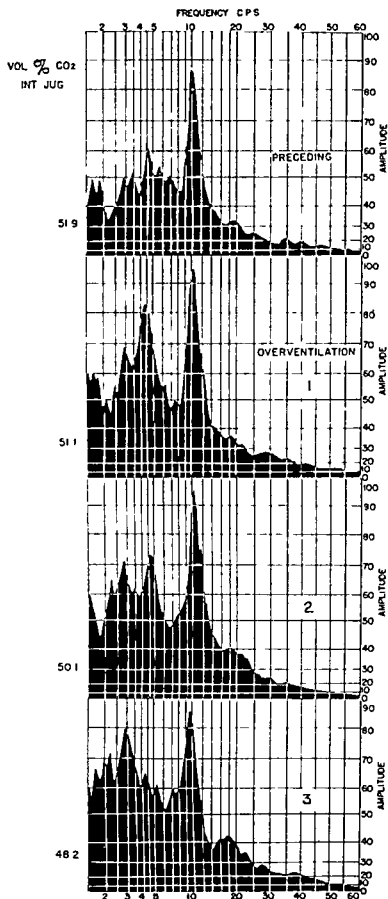
The studies on high and low sugar were done on three normal subjects, those on high and low CO_2 on four normal subjects and those on low O_2 on three normal subjects. The data on high O_2 are based on experiments on four rabbits.

RESULTS

As will be seen in Fig. 1 to 4, slight changes in the CO_2 content of the blood in the internal jugular vein and presumably in the CO_2 tension of the brain are associated with marked changes in the cortical frequency spectrum. These can be described most accurately by saying that as the CO_2 falls,

LOW CARBON DIOXIDE

(SUBJECT WD)



LOW CARBON DIOXIDE

(SUBJECT DW)

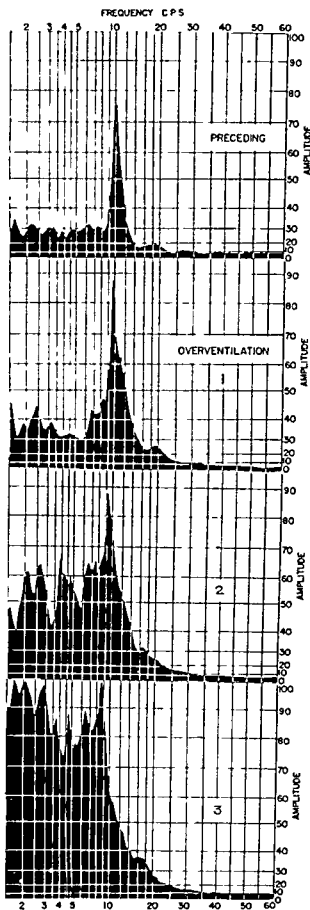


FIG. 1 (Left). A shift of the 19-per-sec. peak to the 18-per-sec. position, and a shift of the 4.5-per-sec. peak toward 4 occurs in this subject with a fall of the CO₂ content of the internal jugular blood from 51.9 to 51.1 volumes per cent. His 10-per-sec. peak is extraordinarily stable. It shifts perceptibly, however, toward 9.5 when the CO₂ content falls to 48.2. With the movement of energy toward the slow side, there is an increase in the total amount of energy in the spectrum.

FIG. 2 (Right). The 10-per-sec. peak in this subject is quite labile, moving further and further to the slow side as the CO₂ is decreased. No blood samples were taken.

HIGH CARBON DIOXIDE

(SUBJECT WD)

HIGH CARBON DIOXIDE

(SUBJECT DW.)

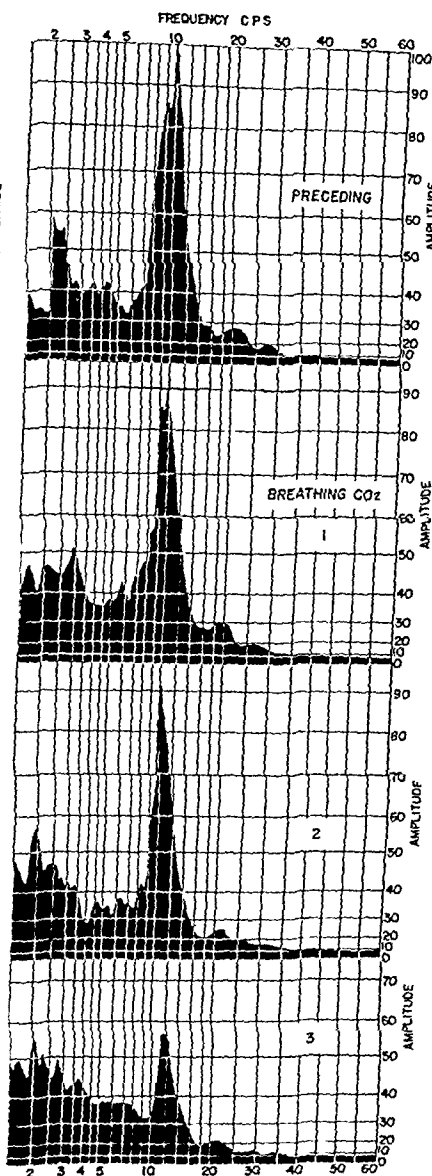
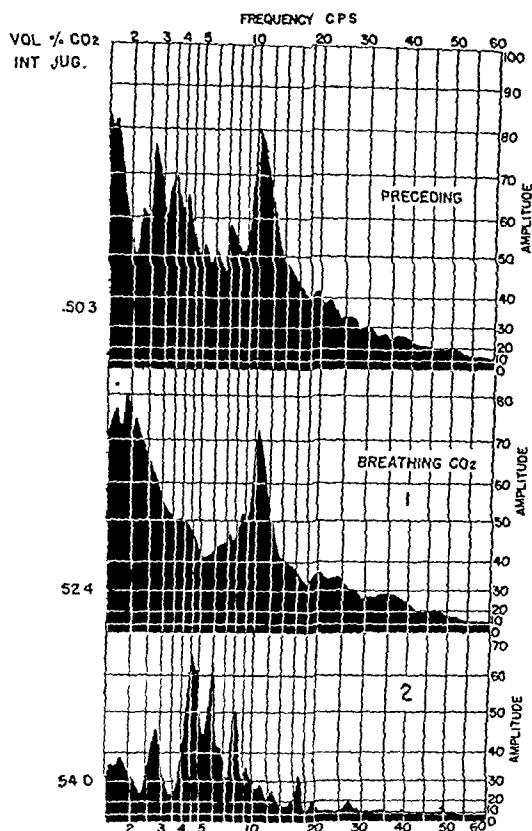


FIG. 3 (Left). The change in the CO₂ content of internal jugular blood from 50.3 to 52.4 is associated in this case with a shift of the 19-per-sec. peak to the 20-per-sec. position the 10-per-sec. peak shifts toward 11, and the peak below 2-per-sec. to a position very close to 2. There is also a relative increase in the energy between 25 and 35 per sec. With a CO₂ content of 54.0, the maximum near 2 becomes lower than the maximum at 2.8. The complex seen at 4, 5.5 and 8 resembles that seen at 2.7, 3.5 and 4 in the first of this series of spectra. The ten peak has disappeared and a definite peak has appeared at 17 per sec. The energy in the spectrum has been decreasing and has shifted toward the fast side. Its maximum has moved from below 2 per second to 2 per sec. and finally to 4 per sec.

FIG. 4 (Right). The gradual shift in this case of the 10-per-sec. peak toward the fast side as the CO₂ is increased is clearly illustrated. No blood samples were taken.

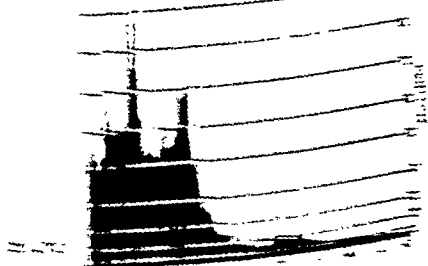
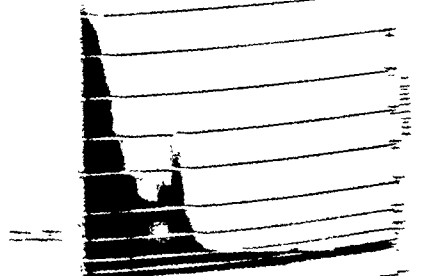
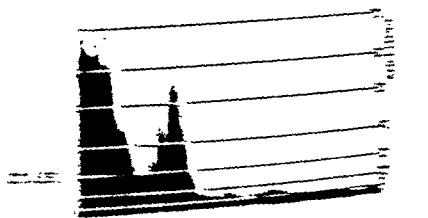
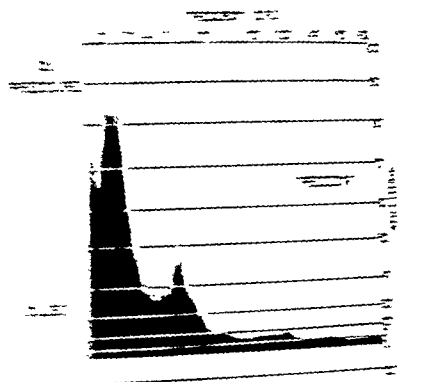
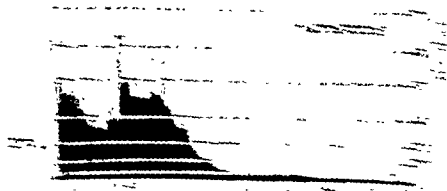
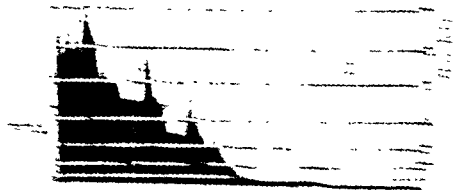
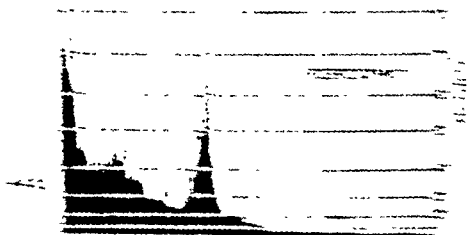
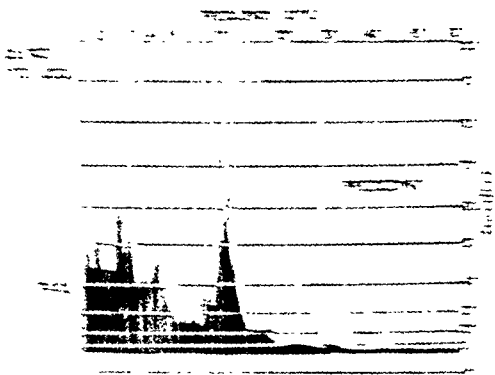
there is a shift in energy toward the slow side of the spectrum with an apparent increase in the total amount of energy. An increase in CO_2 is associated with a shift in energy toward the fast side of the spectrum with an apparent decrease in the total amount of energy. With low O_2 (Fig. 5), there is little change in the cortical frequency spectrum until a level of 40 per cent O_2 saturation in the internal jugular vein is reached, when there is a shift in energy toward the slow side with an apparent increase quickly followed by an apparent decrease in total energy. With high O_2 (Fig. 6), there is little change until an O_2 pressure approximately 35 lbs. per square inch is reached; at this point there is a sudden shift toward the fast side of the spectrum with an apparent increase in the total amount of energy. Low sugar (Fig. 7) produces little change until the blood in the internal jugular vein reaches a level of 30 mg. per 100 cc., when there is a sudden shift toward the slow side with an apparent decrease in the total amount of energy. When the blood sugar is raised, a change occurs which is similar to that seen with high O_2 . At a blood sugar level of 500 mg. per 100 cc., there is an abrupt shift of energy toward the fast side of the spectrum with an apparent increase in the total amount of energy.

DISCUSSION

The level of CO_2 in the internal jugular vein is the only factor which has a definite relationship to the changes which occur in cortical frequency when one overventilates or breathes high CO_2 . It is not known whether the effect of *changes in CO_2 tension on cortical cells is direct or indirect. It may be due to specialized chemo-receptors which indirectly alter the cortical activity.* A mechanism similar to that found in the respiratory center, with its specialized accessory chemo-receptor, the carotid sinus, may be involved. If so, the chemo-receptor in this case must lie somewhere in the brain. That certain elements are more sensitive than others to CO_2 , is altogether likely. Such a specialized chemo-receptor has not been demonstrated, however, so that it is wiser to consider that CO_2 has its effect directly on the cortical cells.

Other workers have spoken of the response of cortical potentials to a decrease in CO_2 as an "increase in activity," but this term is not sufficiently specific to describe what occurs. Nor is it possible to describe these changes in terms of amplitude only, as for example by saying that low CO_2 produces an increase in amplitude. The truth or falsity of this statement will depend upon what frequencies are referred to and the particular level of the CO_2 that is meant. The amount of 10-per-sec. activity in subjects D.W. and W.D. (Fig. 1 and 2) first increases and then decreases as the CO_2 level continues to fall.

The frequencies at which peaks or maxima appear at the fast end of the spectrum are often multiples or near multiples of the frequencies at which peaks appear at the slow end of the spectrum. These peaks may shift independently of one another. A slow peak may disappear, and the peak at



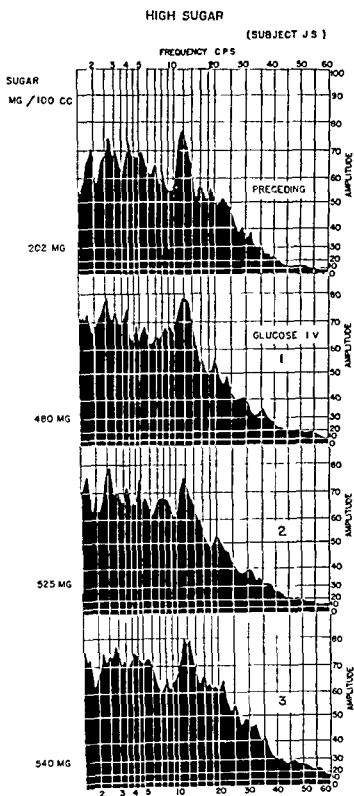
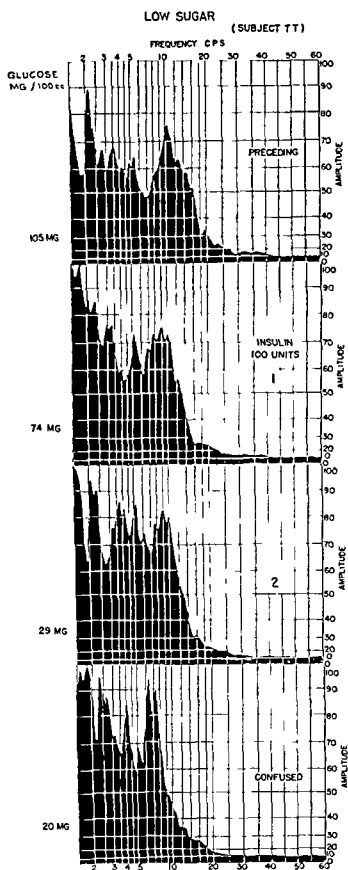


FIG. 7 (Left) With the fall from 105 to 74 mg. of sugar per 100 cc. of internal jugular blood there is evidence of an increase in energy in the slow frequencies and a decrease in energy in the 16-per-sec region, but there is no definite shift in peaks until the sugar level falls below 29 mg per 100 cc. The subject was confused during the 30-sec period covered by the last spectrum.

FIG. 8 (Right). Slight shift to the fast side of the spectrum can be detected when the sugar level is raised from 202 to 480 mg. per 100 cc. of internal jugular blood. A very evident shift in the form of a great increase in the energy in the frequencies above 15 per second occurs when the sugar level is changed from 525 to 540 mg. per 100 cc. This shift toward the fast side is associated with an increase in the total amount of energy in the spectrum.

twice the frequency at which the slow peak occurred may gain in amplitude. Such changes are usually evident in the unanalyzed record as a disappearance of 10-per-sec. waves and the appearance of many more 18- to 20-per-sec. waves, to cite a specific example. *One cannot say what part of the energy at a given frequency is derived from relatively pure sign waves, which would appear as discrete and countable waves of that frequency in the unanalyzed record, and what part is derived from non-sinusoidal slower frequencies, the faster components of which are quite properly recorded as higher frequencies. In any case, it is permissible and profitable to group them together and to talk about the energy delivered at a given frequency as though it emanated from oscillators operating at that frequency.*

In describing the changes occurring in the cortical frequency spectrum, reference has been made to an "apparent" increase in total energy, when there is an increase in the total area under the curve. An increase in area, such as occurs with low CO_2 , does not necessarily indicate an increase in the amount of potential energy being used in the brain, but may indicate only an increase in the efficiency of the oscillators responsible for the electro-encephalogram when they are operated at slower frequencies. This is a characteristic of all oscillators in which an inertia-like factor can control frequency. Rate of diffusion would be such a factor in an electro-chemical oscillator, such as is under consideration here. Likewise, a decrease in amplitude with an increase in frequency, such as occurs with high CO_2 , does not necessarily indicate that less potential energy is being used, but may indicate only that the oscillators are less efficient at high frequencies. A shift toward the fast end of the spectrum, however, with an increase in the total area under the curve, as occurs with high O_2 pressure and with high blood sugar levels, strongly suggests an increase in the total potential energy being used. A decrease in the area under the curve, such as occurs with low O_2 pressure and with low blood sugar, suggests a decrease in the total potential energy being used.

Careful study of a consecutive series of spectra in which energy is gradually shifting reveals that a peak of energy at a given frequency tends to remain more or less constant while the slopes of the peak are pushed further and further in the direction of the force which is operating, for example, to the slow side with low CO_2 . This suggests that the majority of oscillators at that frequency tend to be fairly stable, but that a certain number are more easily disturbed than others. If one considers the hypothetical case in which some oscillators having a frequency of, let us say, 10 per sec. are slowed to 9.5 and others which have a frequency of 10.5 per sec. are slowed to 10, the peak may remain in the same position but the sides of the peak may change. When, however, the force of the factor which is causing the shift becomes sufficiently strong, the entire peak shifts. Its movement appears to be limited, however, for as the main peak moves from, say, 10 to 9.5 per sec., its height rapidly diminishes. *At the same time, the slower peaks gain in height as though they were absorbing the energy lost by the disappearing*

faster peak. In some cases, the frequencies appear to gain more than the diminishing peak has lost, and at times a peak may decrease without much evidence of an increase in the height of a neighboring peak. These observations suggest that there are natural periods at which these oscillators operate most efficiently.

SUMMARY AND CONCLUSIONS

The responses of the electrical activity of the human cortex to alterations in normal blood constituents have been analyzed with the Grass frequency analyzer. Decrease in CO_2 content in the internal jugular blood is associated with a shift in energy distribution in the cortical frequency spectrum toward the slow side; an increase with a shift in energy distribution to the fast side; these effects become less marked with extremely high or extremely low CO_2 tensions. Oxygen and glucose, on the other hand, can be varied within wide limits with little change in the cortical frequency spectrum but when the O_2 saturation or glucose concentration in the internal jugular falls to a critically low level, there is a sudden shift of energy distribution to the slow side. With exceedingly high concentrations of glucose, the energy distribution in the cortical frequency spectrum shifts toward the fast side. Experiments on rabbits indicate that the effects of high O_2 tension are similar to the effects of high concentrations of glucose.

The results suggest that the electrical activity of the cortex is a manifestation of the activity of a great number of chemical oscillators having different natural periods. Though differing in their periods, these oscillators tend to respond similarly to any given factor. A factor which affects one frequency tends to affect all frequencies in the same direction, though not necessarily to the same degree.

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OCULAR ROTATION IN ANESTHESIA AND UNDER THE INFLUENCE OF SUPRANUCLEAR CENTERS

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I. INTRODUCTION

WHILE ocular movements in horizontal or vertical directions have been studied in much detail, very little is known about the influence of supranuclear centers on rotation of the eyeballs. In a study of ocular positions and movements in sleeping individuals, Pietrusky (1922) occasionally observed rotatory movements of the eyeballs. In a case of post-encephalitic hyperkinesia reported by Muenzer (1933), left-sided facial spasm was associated with rotation of both bulbs to the right; there was also slight paresis of the muscles supplied by the left V, VII, and XII nerves and of the left arm. The meagerness of our knowledge on this subject may be partly due to the fact that rotatory movements easily escape observation if one does not direct attention to this phenomenon, for instance by watching vessels in the sclera. Our study of this question was instigated by an accidental observation.

In the course of experiments on the brain-stem of cats, the appearance of a striking oblique position of the pupils was observed indicating an asymmetrical inward rotation of the eyeballs, *i.e.*, a rotation with the upper end of the vertical diameter of the corneas inward, around the axis through the anterior and posterior poles of the eyeballs. In an attempt to analyze this phenomenon, the question presented itself as to how far this rotation was due to the anesthetic (nembutal 35-40 mg. per kg., intraperitoneally). In fact, a similar rotation appeared under the influence of nembutal alone. It seemed, therefore, desirable first to study systematically the effects of anesthetics (experiments on 67 cats.)

METHOD

Before and during the course of anesthesia, the position of the pupil or of a linear scar, burnt into the cocanized cornea as closely as possible to the vertical diameter, was recorded by using a transparent celluloid protractor whose lower edge fitted into and was made movable along a deep groove in a horizontal metal bar fixed to the head-holder, parallel to the mouth.

For a study of the influence of supranuclear centers by stimulation and extirpation experiments, it was always necessary to burn scars into the corneas, since dilatation of the pupils often occurs, and since the pupils may be used as indicators of the ocular rotation only when they are slit-like or oval. To avoid the effect of the anesthetic as much as possible, these experiments were performed in superficial ether anesthesia.

II. RESULTS

The effects of anesthesia are summarized in Table 1.

Table 1. *Effects of anesthetics.*

Anesthetic	Number of animals	Average rotation		Range of rotation		Number of cases showing				
		Right eye	Left eye	Right eye	Left eye	Bilateral inward rotation	Bilateral outward rotation	Bilat. rot. in same direction	No rot. in one eye In. in other	No rot. in one eye Out. in other
Ether	9	0 85° out	0 23° in	6½° out to 4½° in	3½° out to 4° in	3	3	2	0	2
Chloroform	4	0 56° in	0 81° in	5½° out to 8° in	4° out to 7° in	3	1	1	1	2
Gréhan's mixture	6	0 42° out	0 5° out	8½° out to 7° in	7½° out to 7½° in	0	1	3	2	0
Chloralose	7	7 07° in	13. 14° in	2½° out to 30° in	2½° in to 34° in	5	0	1	1	0
Urethane	7	6 14° in	10. 71° in	15° out to 20° in	4½° out to 26° in	5	1	1	0	0
Dial-urethane	6	12 00° in	8 00° out	1½° out to more than 25° in	17½° out to ½° in	1	1	3	1	0
Sodium barbital	6	8 58° in	9 42° in	22° out to 25° in	0° to 30° in	4	0	1	1	0
Sodium phenobarbital	6	12. 67° in	7 67° in	0° to 18° in	5° out to 18½° in	4	0	1	1	0
Sodium pentobarbital	16	5 31° in	5. 36° in	7½° out to 24° in	30° out to 35° in	12	2	3	0	0

The smallest range of rotation (8.5° outward to 8° inward) was noticed under the influence of ether, chloroform, or Gréhan's mixture (5 per cent chloroform in 50 per cent alcohol). A variety of effects could be observed: in- or outward rotation of one eye only or of both eyes, and also rotation of both eyes in the same direction ("homonymous rotation").

The effect of barbiturates (sodium barbital, sodium phenobarbital, sodium pentobarbital, dial-urethane,*) and also of chloralose and urethane was much more marked, the range of rotation reaching from 35° inward to 30° outward.

In the majority of the cases of this second group, the eyes were inwardly rotated (bilateral inward rotation in 31 of 49 observations; inward rotation of only one eye in 4 cases). Much more rarely, bilateral outward rotation appeared (4 instances of bilateral outward rotation of relatively slight degree), or rotation of both eyeballs in the same direction (10 observations). The tendency for homonymous rotation was manifested particularly in dial-urethane anesthesia (3 of 6 cases). In the cases with heteronymous as well as in those of homonymous rotation, it was usually more marked in one eye than in the other.

The extent of the rotation was subject to fluctuation during the anesthesia, e.g., in one stage the right eye might be markedly inwardly rotated and the left only slightly, while at a later stage the opposite might be observed. Often, however, the eyeballs retained the same degree of rotation for several hours. Involuntary horizontal and more rarely vertical movements of the eyeballs (undulation or nystagmoid movements) appeared in some experiments, but the rotation was as a rule independent of these motor phenomena.

Thus, particularly those anesthetics that act mainly on the brain-stem, such as the barbiturates, produce a marked ocular rotation, while so-called cortex anesthetics (Pick and Molitor, 1929), such as ether, chloroform, or chloroform-alcohol show such an effect to a slight degree only. A sharp division between cortex- and brain-stem anesthetics seems, however, impossible since a so-called cortex anesthetic (chloralose) may produce a marked bilateral inward rotation.

For further analysis of these phenomena, it seemed of interest to study the influence of various parts of the central nervous system upon the position of the eyeballs with special reference to rotation around the antero-posterior axis of the eye (experiments on 25 cats).

Stimulation of the cortical centers for ocular movements in the frontal or occipital lobe produced, besides conjugate deviation of the eyeballs to the opposite side and less frequently vertical deviation, a slight rotation in the majority of the experiments (Table 2). The most frequent reaction was a rotation of the opposite eye towards the stimulated hemisphere (in regard to the upper end of the vertical diameter of the cornea, average less than

* The dial was kindly supplied by Ciba Pharmaceutical Products Inc.

5°, maximum 15°). The reaction of the homolateral eye was more variable. The inward rotation of the opposite eye might be accompanied by a slighter inward rotation of the homolateral eye; or the homolateral eye might rotate outwardly so that both eyes rotated towards the stimulated cortex; or a reaction of the ipsilateral eye might be absent. In a few instances, rotation was observed in the homolateral eye only (outward rotation), or this reaction was accompanied by a weaker outward rotation of the opposite eye. Extirpation of the frontal or occipital oculogyric centers or combined elimination of these areas resulted only in a slight and temporary inward rotation reaching not more than a few degrees. Thus, a definite tonic effect of the cortex upon the position of the eyes in regard to rotation could not be found.

Table 2. Stimulation of cortical oculogyric centers.

contralateral eyeball	Rotation of ipsilateral eyeball	Number of observations
no rotation	no rotation	12
inward (to stimulated cortex)	inward (to non-stimulated cortex)	10
inward (to stimulated cortex)	outward (to stimulated cortex)	7
inward (to stimulated cortex)	no rotation	5
no rotation	outward	2
outward	outward	4
		40

After combined extirpation of the prosencephalon and diencephalon by a section in front of the midbrain and after discontinuation of the ether anesthesia, inward as well as outward rotation of one or both eyeballs could be observed (5 times both eyes inward, twice both eyes outward, once homonymous rotation, once no rotation). Only in 3 out of 9 experiments was the vertical diameter of the cornea rotated by more than 10° from the vertical position; the maximum rotation observed was 20° inward rotation on the left and 2.5° inward rotation in the right eye. In this case the anatomic examination revealed that the section traversed the brain-stem dorsally in front of the superior colliculi, ventrally just in front of the chiasma, while in the other cases the ventral end of the section was just in front of the cerebral peduncles or through the tuber cinereum.

Such high degrees of rotation as were obtained under the influence of such anesthetics as the barbiturates and chloralose were, however, not reached in these extirpation experiments. This may be partly due to the fact that the tone of the mesencephalic ocular nuclei may become impaired after section in front of the midbrain as a consequence of shock, as indicated by a more or less pronounced dilatation of the pupils. Furthermore, the possibility has to be borne in mind that the rotation in anesthesia may be produced

not only by elimination of the action of higher centers upon the mesencephalic ocular nuclei but also by a direct action of the anesthetics upon parts of the brain-stem below the diencephalon. For dial, an action upon the oblongata is suggested by such symptoms as salivation, vomiting, and occasional respiratory failure (Fulton, Liddell, and Rioch, 1930).

In our observations, an action upon the rhombencephalon, particularly the vestibular nuclei, is suggested by cases of anesthesia with marked homonymous ocular rotation, which is usually of unequal degree in both eyes. This homonymous rotation may reach higher degrees (up to 25° in unoperated animals) than that observed in the above-mentioned experiments with extirpation of prosencephalon and diencephalon. Now it is known that a marked homonymous rotation to the side of operation appears after unilateral labyrinthectomy (Rademaker, 1935). Godlowski (1938) observed homonymous rotation to the stimulated side on stimulation of the most cranial parts of the posterior longitudinal fasciculus, leaving it undecided whether this effect was due to ascending or descending fibers.

Our experiments showed that the rotation to the operated side following unilateral labyrinthectomy is more developed in the opposite eye (average 20°) than in the homolateral eye (average 7°). A similar type of rotation may be obtained by a unilateral lesion of the vestibular nuclei (incision on the inner side of the restiform body). The rotation may still be observed after the nystagmus following the labyrinthectomy subsides, and also without accompanying nystagmus on a lesion of the vestibular nuclei in anesthetized animals. Thus, the fact that the homonymous rotation in anesthesia may appear without nystagmus does not speak against the possibility that the effect of the anesthetics may reach in these cases the rhombencephalic vestibular nuclei disturbing the balance between the nuclei of the two sides.

In order to test the possibility of a direct action upon the centers behind the thalamus, transverse sections immediately in front of the superior colliculi and the cerebral peduncles were performed in ether anesthesia, which was discontinued after this operation. Some time later, after the position of the eyeballs in regard to rotation had become fairly constant, a brain-stem anesthetic (usually dial-urethane) was injected intraperitoneally. While in some experiments the change in degree of ocular rotation was only slight and transitory, in others it was marked (maximum in one case was 11° outward, in another case 17.5° inward) and lasted for hours. This corroborates the assumption of a direct action of the anesthesia upon parts of the brain-stem behind the diencephalon.

An attempt was made to ascertain whether anesthetics may still produce ocular rotation if the mesencephalon is separated from the supranuclear rhombencephalic centers. After ocular rotation had developed in otherwise normal animals under the influence of barbiturates (nembutal, dial-urethane), a transverse section through the most cranial part of the pons was performed. Such a section diminished the rotation produced by the anesthetic but failed to abolish it, while the contraction of the pupils indicated

that the mesencephalic eye muscle nuclei were still in a good condition (e.g., inward rotation in nembutal anesthesia on the right eye 12–15° before, 10–13° after the section; on the left eye 8–12° before, 5–7° after the section; pupil diameter on the right side 3 mm. and on the left side 4 mm. after the section). Similar observations were made in mid-brain animals in favorable cases (e.g., bilateral outward rotation produced by dial-urethane was diminished but not abolished by transverse section behind the posterior colliculi sparing the basilar artery). Thus on one hand cases of homonymous rotation suggest that the action of the barbiturates may reach as far down as the vestibular nuclei; on the other hand it could be shown that an action upon rhombencephalic supranuclear centers is dispensable, and that a direct action upon the mesencephalon also exists, particularly in the production of heteronymous rotation.

Ligature of the carotid arteries, which procedure, according to de Kleyn and Versteegh (1922), keeps toxins circulating in the blood away from the eye muscles, did not prevent the appearance of ocular rotation under the influence of anesthetics.

SUMMARY

1. Under the influence of anesthetics, particularly of the so-called brain-stem anesthetics, various types of ocular rotation (heteronymous and homonymous rotation) occur.

2. Stimulation of the cortical oculogyric centers may produce, besides conjugate deviation in a horizontal or vertical direction, a slight rotation (most frequent reaction: rotation of the opposite eye towards the side of stimulation). Tonic effects of the cortex upon the position of the eyeballs in regard to rotation could not be found. Elimination of the cortex plus subcortical ganglia in front of the midbrain produces moderate degrees of rotation, but not such high degrees of rotation as are observed under the influence of anesthetics.

3. After unilateral labyrinthectomy, rotation to the side of the operation is more marked on the opposite side than on the homolateral eye; this rotation may outlast the spontaneous nystagmus. A similar homonymous rotation may be produced by a unilateral lesion of the vestibular nuclei, suggesting that disturbances of the equilibrium between the vestibular nuclei of the two sides may play a part in the genesis of homonymous rotation observed in barbiturate anesthesia.

4. Ocular rotation is still produced by brain-stem anesthetics such as dial-urethane in mid-brain animals. It is inferred that the ocular rotation produced by anesthetics is only partly due to depression of prosencephalic and diencephalic activity and partly to direct action upon the lower centers.

5. Separation of the mesencephalon from the rhombencephalon in normal as well as in mid-brain animals diminishes the rotation produced by barbiturates, but does not abolish this rotatory effect; this indicates a direct action of the anesthetic upon the midbrain, besides the action upon rhombencephalic supranuclear centers.

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EFFECT OF VARIOUS CORTICAL LESIONS ON DEVELOPMENT OF PLACING AND HOPPING REACTIONS IN RATS

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REMOVAL of the sensorimotor areas of the cerebral cortex from a variety of mammals causes permanent deficiencies in placing and hopping responses directly proportional to the degree of cortical control characteristic of the particular species (Bard, 1933; Brooks, 1933; Woolsey, 1933; Brooks and Woolsey, 1936; Woolsey and Bard, 1936). Even in rats the control of placing reactions is so strictly localized in the sensorimotor cortex that no observable recovery of these functions occurs following ablation of that area. These placing and hopping responses, first described by Rademaker (1931), are not present in most mammals at birth. Several days are required for their full development. Yueh Tang (1936) has described the time and order of their appearance in rats and has also endeavored to correlate development of responses with phases of cerebral maturation.

In higher mammals certain changes resulting from cortical injury gradually disappear and deficiencies which are obvious immediately following cortical ablations become less apparent after several weeks (Hines, 1937). It is claimed by Kennard (1936, 1938) that a much greater degree of readjustment or recovery occurs in monkeys if lesions are made in infancy. No detectable improvement in hopping and placing responses of adult rats was observable (Brooks, 1933) after these reactions had been rendered deficient (hopping) or abolished (tactile placing) by complete ablation of sensorimotor cortex. Hopping responses which are elicited by adduction, abduction or retroflexion of a leg are presumably initiated by proprioceptive stimuli. In adults, after removal of the sensorimotor areas hopping still occurs, but a greater degree of displacement is required for initiation of the reaction, i.e., the response is rendered deficient, but not abolished. Placing reactions which occur when the vibrissae or the hairs of a foot or toe come in contact with an object are the result of tactile stimulation. These tactile, or contact placing responses are permanently abolished in the adult by ablation of the sensorimotor areas of the cortex. The question arises whether or not these abnormalities in response would result if the sensorimotor areas were removed from newborn rats before development of placing and hopping reactions. Possibly under these conditions other regions of the maturing cortex, or subcortical structures, would be capable of assuming a function normally dependent on the sensorimotor area.

47.344

METHODS

Eleven litters containing a total of 55 rats were used in this study. Preliminary operations were performed before development of the hopping and placing responses, *i.e.*, from the 1st to the 5th day after birth. The young animals were anesthetized with ether and various portions of the cerebral cortex were removed by means of a capillary pipette and gentle suction. These animals were observed daily from time of operation to time of autopsy and were found to be so slightly affected, even by lesions as extensive as hemidecortication or bilateral removal of the frontal third of the neocortex, that they had little difficulty competing with litter mates for food. Once each week during the month following operation the rats were tested for evidence of hopping and placing responses. The tests used in examining these animals were similar to those described by Brooks (1933). Two months after operation one of us, not knowing the site of the cortical lesions, made a detailed examination of each animal. On the basis of the type of deficiency observed predictions were made concerning the locations of the lesions. At this time either the sensorimotor or various other areas were removed from the intact hemispheres of ten of these animals which had been subjected previously to a unilateral ablation. We were able, in this way, to compare in the same animal effects of operations performed before and after appearance of placing and hopping reactions.

Two to 6 months later all animals were again tested. Following this final examination, the cortices of 19 of the rats were stimulated electrically. The extent of the lesions in the sensorimotor area and the electrical excitability of various cortical remnants were thus ascertained. The stimulator employed delivered a 60-cycle sinusoidal wave. Voltage and current strengths were recorded simultaneously during stimulation. A unipolar electrode was used for exploration of the cortex, the indifferent electrode being placed on the abdomen. The remaining rats were autopsied and their brains studied.

RESULTS

Our study of the development of placing and hopping responses of young rats revealed that although there is considerable variation in speed of maturation, reactions develop in an orderly sequence within ten days to two weeks after birth. The conclusions of Yueh Tang (1935) concerning the order and time of appearance of placing and hopping responses were thus confirmed. This development is apparently little, if at all, retarded by removal of electrically inexcitable areas of the cortex. Likewise, removal of a portion of sensorimotor cortex does not retard the appearance of leg reactions controlled by remaining portions of the area. Ablation of one hemisphere does not prevent normal development of these responses in limbs contralateral to the intact hemisphere.

Six animals which had undergone complete ablation of the sensorimotor area of one hemisphere, between 1 and 5 days after birth, failed to develop normal hopping and placing reactions in limbs contralateral to the lesion. Bilateral removal of electrically excitable cortex (one animal) shortly after birth resulted in maximal bilateral deficiencies; this animal never developed tactile reactions and hopping responses which did appear were as deficient as those of decorticate adults. Lesions involving not only the sensorimotor areas, but, in addition, other portions of the cortex, failed to cause detectably greater degrees of deficiency. The deficiencies of 4 hemidecorticate rats were indistinguishable from those of animals whose lesions included only the sensorimotor cortex of one hemisphere.

Deficiencies resulting from removal of the entire sensorimotor area during infancy appeared to be as great as those following removal of comparable

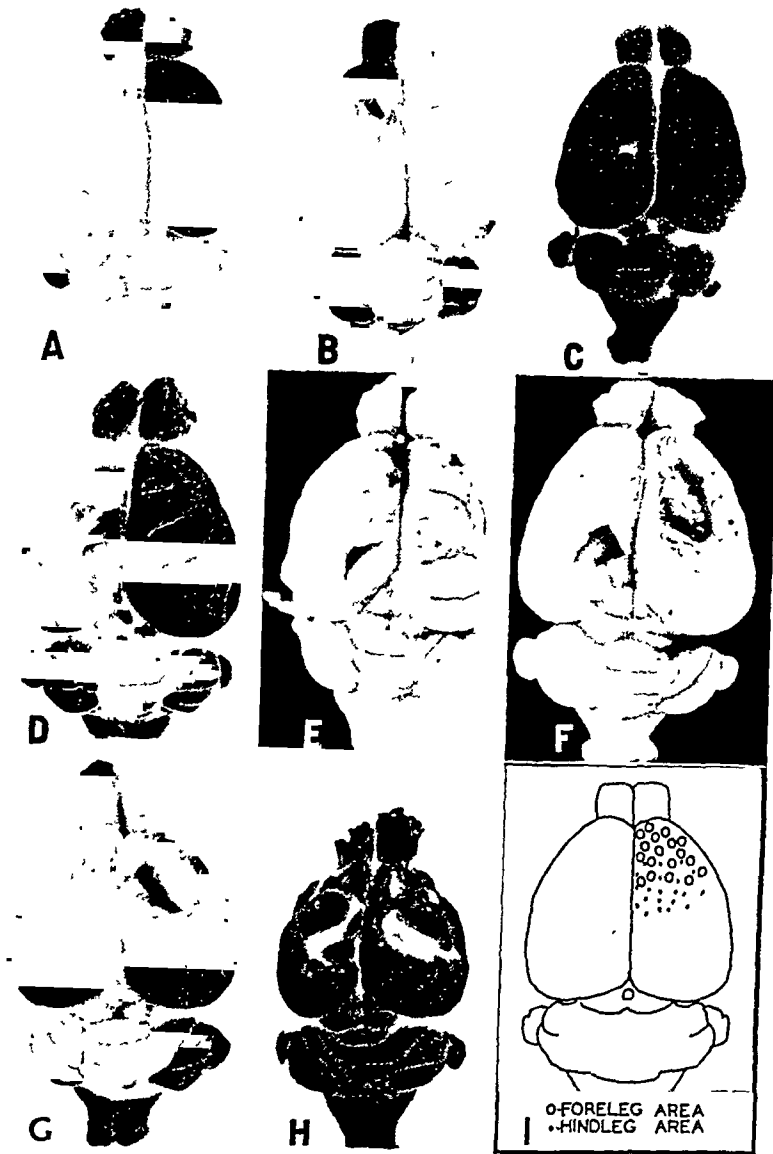


FIG. 1. A. A small cortical lesion made twelve days after birth which caused permanent, but barely detectable, deficiencies in reactions of contralateral foreleg.

B. A lesion which produced easily detectable deficiencies in the contralateral foreleg, but no apparent deficiencies in the reactions of contralateral hindleg. Lesion made five days after birth.

C. The reactions of both right fore and hindleg were rendered slightly deficient by this small lesion. The hindleg responses were affected to a greater degree than were those of the foreleg. No recovery was observed although the lesion was made two day after birth.

D. A lesion in the occipital region of the cortex which included a considerable portion

areas of adult rats. Five animals which had undergone unilateral removal of the sensorimotor area between days 1 and 5 were operated upon a second time, 15 weeks later. In this way we were able to compare, in the same animal, the permanent effects of ablation of the area shortly after birth and after maturation of all responses. No differences in deficiencies on the two sides could be detected in these preparations 2 months after the second operation (Fig. 1H).

In 36 young rats lesions were made which involved only portions of the electrically excitable areas of the cortex. These lesions varied in size and location. In 1 animal the lesion caused no detectable deficiency and in 3 any abnormalities of reaction, if present, were very slight after 5 months. At autopsy, minute lesions were found in the sensorimotor cortex. Stimulation of the cortex, moreover, showed that only a small fraction of the electrically excitable area had been removed.

Three mature rats which had been operated on during infancy showed impairment of the responses of the contralateral foreleg, but the hindleg responses were apparently normal. At autopsy, it was found that the cortical lesions were located within the foreleg area of the sensorimotor cortex (Fig. 1A and B). A few of these animals showed normal foreleg activity, but deficiency followed a small lesion confined to the hindleg area of the sensorimotor cortex. In 3 others a large occipital lesion had involved a portion of the hindleg area (Fig. 1D). Stimulation of the anterior margins of these lesions caused hindleg movements, but it was obvious that a considerable portion of the hindleg cortex had been ablated.

Twenty-eight rats with small unilateral cortical lesions showed a degree of deficiency in reactions of both opposite legs. When these rats were compared with hemidecorticate rats, or with others having complete removal of unilateral sensorimotor area, it appeared that the legs were not maximally

of the hindleg area. Permanent deficiencies in the reactions of the contralateral hindleg resulted. Lesion made five days after birth.

E. A lesion in the occipital cortex which caused no detectable deficiencies in hopping and placing responses of the contralateral hindleg.

F. The lesion in the left cortex (made five days after birth) resulted in deficiencies which were confined to the contralateral hindleg. The lesion in the right hemisphere, made 54 days later, caused maximal deficiencies in the left legs. Right foreleg movements were elicited by electrical stimulation of that portion of the left hemisphere anterior to the lesion. No leg movements could be obtained by stimulating other portions of the cortex of either hemisphere.

G. The lesion in the left hemisphere (made five days after birth) rendered the right foreleg clearly deficient. The reactions of the right hindleg were affected to a lesser degree. Ablation of the sensorimotor cortex of the right hemisphere when the animal was six weeks old rendered the left legs more deficient than were the right. Stimulation of the cortex of this brain revealed that remnants of foreleg and hindleg areas remained in the left hemisphere.

H. The lesion in the left hemisphere was made two days after birth; that in the right hemisphere two months later. The deficiencies in the placing and hopping responses resulting from the two lesions were identical.

I. Electrically excitable areas as determined by stimulation of the right hemispheres of eleven rats.

deficient. To test this the sensorimotor cortex of the intact hemisphere was ablated. The legs contralateral to the new large lesion were more deficient than those contralateral to the smaller and older lesion. At the termination of these experiments exploration of the cortex containing the smaller lesion revealed the presence of a little tissue of both foreleg and hindleg areas (Fig. 1G). It was concluded that these areas were of some functional importance and were responsible for differences in the degree of deficiencies of the two sides.

In 5 rats, lesions made in infancy in the occipital cortex or other non-excitabile areas did not result in deficiencies of response (Fig. 1E) at any time during survival periods of 6 months. Stimulation of the cortex of these animals showed a normal presence and distribution of electrically excitable areas. Figure 1-I is a composite diagram of the points from which electrical stimulation elicited foreleg and hindleg movements in eleven rats. These experiments show that removal of sensorimotor cortex from young rats before development of placing and hopping responses does effect the reactions permanently. No other areas were able to assume control of these responses. Even small lesions within these areas result in detectable permanent deficiencies.

On the basis of the deficiencies of each of these 55 animals, the locations of lesions were predicted. At autopsy it was found that only two serious errors in prediction had been made. In 9 additional cases the prediction was partially incorrect. That is, in these animals which showed some deficiencies in both fore and hindleg response, mistakes were made when we attempted to judge whether the larger part of the lesion was in the fore or hindleg area.

Examination of the pyramidal tracts above the decussation, revealed a few points of interest. Those animals in which a small lesion had been made in the foreleg or hindleg area showed a reduction in size of the bundle ipsilateral to the lesion. In those animals in which an occipital lesion had encroached upon the hindleg area, there was a smaller pyramid on the ipsilateral side. Occipital lesions which did not impinge upon sensorimotor cortex did not detectably modify the pyramid. In those animals in which deficiencies were not maximal pyramidal fibers were sufficiently numerous to be detected macroscopically. The reduction in size was roughly proportional to the magnitude of the sensorimotor lesion. Completely hemidecorticate animals, and those in which the frontal portions of the cortex (sensorimotor area) had been removed, possessed no macroscopically visible pyramid ipsilateral to that hemisphere. Histological study of some of these brains, however, revealed a few fibers which might have been incorporated in that tract.

DISCUSSION

The placing and hopping reactions are maximally developed in adult rats but some such as the hopping response to adduction of a leg, can be rendered

only slightly abnormal by removal of the cortex (Brooks, 1933). No difficulty was encountered in determining the presence or absence of contact placing and the other responses which were absent after complete removal of the sensorimotor area, but the degree of deficiency of hopping reactions was harder to judge. Small rats were more active than adults and some difficulty was encountered in studying their responses. Consequently, a great deal of variation was seen, but it may be explained as due either to fluctuations in emotional state of the animal, *i.e.*, the degree of excitement, or to the manner in which the animal was held, *i.e.*, in painful or uncomfortable positions. Nevertheless, even in young animals, it was possible to determine slight deficiencies.

It can be concluded that a small lesion within the foreleg or hindleg cortical area produces detectable abnormalities of a permanent nature, even when the operation is performed on the first postnatal day. The entire foreleg or hindleg area must be ablated, however, before the state of maximal deficiency in these reactions is attained. The maximal deficiency is that state which results from complete decortication or complete removal of the frontal half of the neocortex.

Incomplete removal of the sensorimotor area was followed by lesser degree of permanent deficiency than was complete ablation of the area. The degree of deficiency was estimated in this way. It was seen that the responses of legs contralateral to injured cortex were deficient when compared with the responses of legs contralateral to the intact hemisphere, but the reactions of the affected legs of some rats were more easily elicited, more prompt and more uniform than those of other rats. This difference might be explained on the basis of individual variations, but a second method of comparison proved this to be an inadequate explanation. In several of those rats which clearly showed contralateral deficiencies after a first operation, but whose responses were definitely superior to those of animals with larger lesions, we ablated the entire sensorimotor cortex of the intact hemisphere. The legs contralateral to this lesion became permanently more deficient than were the legs opposite the smaller original lesion. This second method of comparing deficiencies gave even stronger proof than the different degrees of deficiency resulted from unequal subtotal lesions. When the original lesion included the entire sensorimotor cortex of one hemisphere removal, from the mature rat, of a comparable area of the other hemisphere resulted in deficiencies of the postural reactions which were equal on the two sides.

Brooks (1933) found that removal of the sensorimotor areas from the cortex of the adult rat produced permanent deficiencies in the postural reactions of the legs contralateral to the lesion. The experiments reported at the present time justify the statement that if any recovery occurs after partial or complete ablation of sensorimotor areas it is never sufficient to restore the placing and hopping responses to normal, even when the lesions are small and are made within a few hours after birth. It is more difficult to determine whether or not partial recovery of these placing and hopping responses takes

place after they have been rendered deficient by ablations involving only a part of the sensorimotor cortex. The infant rat has none of these postural reactions and, consequently, only the ultimate state of deficiency can be ascertained. In the adult rat and in young rats, which have developed the placing and hopping responses, all reactions are somewhat depressed immediately following ablation of a portion of the cortex. The placing and hopping deficiencies are more noticeable then than they are a few hours later. It is conceivable that this temporarily greater impairment of response is due entirely to after effects of the anesthetic, mild surgical shock or to such temporary conditions as irritation, edema, or an impairment of circulation in the subcortical structures. In the original studies by Brooks (1933) it was concluded that improvement in the responses is probably due to general recovery of normal activity rather than to an assumption of a new function by the remaining tissues. The temporary depression observed in the reactions of limbs opposite intact cortex was likewise interpreted to be an indication of a general depression of all activity rather than an indication of an ipsilateral effect of the ablation. This study of young animals has furnished no evidence which would justify a modification of this conclusion.

It also seems reasonable to assume that another reason why the animals appear to be more deficient immediately after operation than they are a few hours later is that readjustments of a complex nature occur. The animals probably learn to avoid movements and reactions which they are unable to execute properly. They use other methods and mechanisms for attaining the same end. This might be called readjustment rather than recovery, if by recovery is meant the reappearance of a reaction which involves the use of the same muscle groups, and if the term recovery implies that another portion of the brain has assumed a function normally executed by the sensorimotor cortex.

The results of this work justify two general conclusions. First, the cortical control of the placing and hopping reactions is strictly localized to the sensorimotor cortex. Even within this general area there is at least some subdivision of function. These localized areas are of paramount importance to any reaction pattern involving postural adjustments. Secondly, no subcortical structure and no other portion of the cortex can assume, even during infancy, this control of the placing and hopping which is normally localized in a particular portion of the sensorimotor area.

SUMMARY

Complete removal of that area of the cortex which in rats 1 to 5 days old corresponds to the sensorimotor area of the adult results in a permanent deficiency of the placing and hopping responses. This deficiency is indistinguishable from that caused by a similar operation on the other hemisphere of the same rat after it had reached maturity.

Small lesions confined to the foreleg or hindleg areas produce permanently detectable deficiencies in these postural reactions. It is possible to

determine the position and extent of the lesion by a study of the deficiencies in the placing and hopping reactions. The deficiencies following incomplete ablation of the sensorimotor area are not as great as those resulting from complete removal of this portion of the cortex.

There is no detectable improvement in the placing and hopping reactions after they have been rendered deficient by removal of the sensorimotor cortex. Even when lesions are made in the sensorimotor cortex immediately after birth, the remaining cortical and subcortical tissues are unable to assume, as they mature, the function normally executed by that portion of the sensorimotor cortex which has been ablated.

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ACTIVITY OF ISOCORTEX AND HIPPOCAMPUS: ELECTRICAL STUDIES WITH MICRO-ELECTRODES

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INTRODUCTION

It is clear that methods are needed for dissecting, physiologically, the electrical activity of the nerve centers, because it is only by limiting to one or a few the number of units which are contributing appreciably to the activity recorded that one may make clear-cut deductions about the processes occurring in the units. This has been attempted in the present experiments by investigating the electrical activity of structurally favorable regions of the brain by means of very small recording electrodes such as have been used in a few previous investigations of nerve and muscle (Gelfan and Bishop, 1932; Hogg, Goss, and Cole, 1934; Barron and Matthews, 1935; Jasper, 1936; Buchthal, 1937; Buchthal and Lindhard, 1937; Dusser de Barenne and McCulloch, 1938). In some of the experiments the choice of a type of anesthesia which results in relatively simple electrical patterns has aided the analysis.

In the interpretation of the present results and the data of the literature, validity is assumed for the following propositions which follow from the potential theory (cf. Pierce, 1902).*

(a) The potential at a point in a conducting medium due to the existence of activity in portions of a cell which is at a distant part of the medium is small (either approaches or actually is zero as the distance of the point from the cell increases). Therefore an electrode remote from a nerve center may be considered as indifferent with regard to electrical activity in that center.

(b) There is no external electrical field set up and therefore no current flows in the medium about a cell so long as every part of the surface of the cell is at the same potential. Even if the potential difference between the

* Serious deviations might occur if barriers or laminae of high impedance were interposed in various positions within the parts of the nervous system under consideration; in our experiments the brain has proved sufficiently homogeneous to preclude appreciable discrepancies. These propositions, of course, represent no original contribution for, as Lorente de N6 (1939) has recently pointed out in an excellent statement of the subject, potential theory was applied to the study of currents of action and injury by Helmholtz as long ago as 1852. We have found the data presented by Craib (1927, 1928, 1930) and by Bishop and Gilson (1929) most informative; the monograph of Wilson, MacLeod and Barker (1933) has also been useful. Our interpretations have been in terms of the membrane theory, although, as Helmholtz indicated, examination of the electric field in the medium about cells does not uniquely define the mode of origin of the loci of the sources of potential difference. Some recent data suggest the possibility that some of the voltage differences recordable from organisms may be due to concentration gradients arising from the diffusion of metabolites in the milieu surrounding cells (cf. Burr and Northrup, 1939; Teorell, 1935).

outside and the inside of the cell should change greatly, no external field would be set up if the change took place uniformly and simultaneously over the entire surface.

(c) An external electric field is set up when a potential difference occurs between any two regions on the surface of the cell. Current flows from the regions of high potential to those of low through the surrounding medium and returns within the cell. Points in the medium close to a region of the cell surface which is at a relatively low potential are negative to the distant point p ; points close to a region which is at relatively high potential are positive to the point p .

(d) The difference in potential between any point n near the cell and a distant point (which is at approximately average or zero potential) due to the condition described in (c) decreases very rapidly as the distance of n from the cell increases. The density of current becomes rapidly greater as an active portion of a cell is approached, because of the decreasing volume through which the action current may flow; consequently, assuming approximately constant specific resistance of the medium, the IR drop becomes rapidly steeper as the active region (or an adjacent inactive region) is approached.

The basis for one valuable use of micro-electrodes is revealed in these propositions, and particularly in (d). It follows that, in records of the potential differences occurring between an indifferent electrode and a micro-electrode placed in a region where active units are randomly distributed, the potential changes contributed by units which have an active region (or an adjacent inactive region) very close to the micro-electrode will be very much greater than those contributed by units farther away. Therefore as an approximation the record consists largely of potential changes due to units close to the micro-electrode, and the volume of tissue contributing effectively to the record is greatly reduced. The data presented below verify the two corollaries, that (1) micro-electrodes should record in a localized way under certain circumstances and (2) large potential differences should be recorded by micro-electrodes placed in certain positions in active tissue. A second use of very small electrodes is the determination of the spatial distribution of potential in a mass of tissue without the infliction of much damage. Our results demonstrate that particularly interesting deductions may be made from such data obtained in regions of the nervous system where the spatial arrangement of cells is particularly simple, as it is in the hippocampus.

Preliminary reports of some of our findings have been presented previously (Forbes, Renshaw and Rempel, 1937; Renshaw, Forbes, and Drury, 1938; Renshaw and Forbes, 1938).

METHODS

Micro-electrodes devised for recording potential changes of low (*i.e.*, physiological) voltage in tissues should possess a few characteristics. First, they should be as small as possible to obtain as large a signal-to-noise ratio as possible. Since this ratio is an inverse function

of the resistance of the input circuit of the amplifier, low electrode resistance increases the resolution of the recording system.

Unlimited resolution of small electrical variations is not possible in spite of the tremendous voltage and current amplifications made available by the vacuum tube (Schottky, 1918; Johnson, 1928; Nyquist, 1928; Llewellyn, 1930). For a properly designed amplifier used with an input circuit of high resistance, the thermal noise originating in the resistance is the factor which mainly limits the value of the ratio, signal-to-noise, for small signals. The thermal noise-level—that is, the magnitude of the spontaneous, fluctuating potential changes measured across the ends of any resistance—depends only on three variables, the absolute temperature of the conductor, its resistance, and the frequency-band to which the recording instrument is sensitive, and varies with the square root of each of these quantities. For the study of physiological transients the frequency-band should extend from approximately zero to about 10,000 cycles per sec., and the temperature is not amenable to reduction. Thus the resistance of the input circuit becomes the only factor which may be altered to reduce the noise-level of the recording apparatus. Using the amplifier and cathode ray tube, noise-levels have been determined for various values of the total resistance of the input circuit as shown in Table 1; the values seem large because they

Table 1

Total resistance (M Ω)	Noise-level (width of baseline in μ V)
0.1	15
1.0	45
10.	150

Table 2

Electrode resistance (M Ω)	Grid resistance (M Ω)			
	0.5	1.0	10.	100
0.01	9.90	9.95	10.0	10.0
0.1	2.88	3.02	3.15	3.16
1.0	0.577	0.707	0.953	0.995
10.	0.0686	0.0954	0.224	0.301

represent more nearly peak-to-peak than the usual root-mean-square values. Since the grid resistor of the amplifier is in parallel with the electrodes, reduction of the grid leak reduces the noise-level, but it also reduces the size of the signal as recorded. Table 2, giving relative values (calculated for small signals and considering only thermal noise in the input circuit) for the ratio, signal-to-noise, as electrode resistance and grid resistance are varied, demonstrates that when high-resistance electrodes are used the degree of resolution obtainable varies inversely with the electrode resistance and directly with the grid resistance. The decreased resolution accompanying the use of high-resistance micro-electrodes is compensated for by the surprisingly large potentials often recorded with them.

Other desirable characteristics in micro-electrodes are electrical stability, non-polarizability and small size; in order to minimize injury to the tissue into which the micro-electrodes are inserted, their inert walls should be as thin as is compatible with proper strength and insulation and their taper back from the small tip should not be greater than considerations of rigidity and electrical resistance demand.

The available micro-electrodes fall into two groups. In one a metal surface of small area is in contact with the tissue (cf. Taylor and Whitaker, 1927); in the other type the lead to the tissue is made by a salt bridge which is microscopic in area at the tip, and the metal interface of the half-cell may be large (cf. Ettisch and Peterfi, 1925; Gelfan, 1927). A type of micro-electrode satisfactory for the present purposes has proved to be of the second group. It is a silver-silver chloride Ringer-agar half-cell in which a chlorided silver

wire dips into a glass micropipet filled with a gel of agar in Ringer's solution (Fig 1). * The tip diameters have varied upward from $15\ \mu$ and the wall thicknesses have been but a small fraction of the whole. Since tissue fluid and Ringer's solution have approximately the same ionic composition, diffusion potentials at the pipet tips must be small. Further there is no danger of diffusion from the pipet of ions which might change or affect the excitability of tissues, this is a danger when the salt bridge is of potassium chloride. The two large sources of potential are the metal interfaces, the relatively large area of the metal surface and the fact that the fluid surrounding it is protected from movements make for stable potentials, and the large surface covered with an excess of silver chloride makes for a relatively high degree of non-polarizability. Repeated tests with electrodes of this sort have demonstrated that, as used in these experiments, they distort neither fast nor slow potential changes of the amplitude actually recorded from the brain, and that they do not show spontaneously fluctuating potential changes. A disadvantage is their resistance—a $40\ \mu$ electrode often has a resistance of 0.5 to 1.0 M Ω .

It is desirable to determine what potential differences occur between two points very close together in the brain. A simple way to be certain that two micro electrodes embedded in tissue actually are a given small distance apart is to have them fastened together as a pair (see diagrams, Fig 5). The two half-cells forming each pair have been the same as described above for single electrodes. Two pieces of capillary tubing are fastened together with de Khotinsky cement. They are partially fused together with a microflame and then pulled out to microscopic dimensions. After breaking off both at the desired length, one is chipped away as far back as is desired, this is done with a fine needle under the dissecting microscope. The dimensions of the pipets of the smallest pairs yet made have been about $25\ \mu$, larger sizes are more easily made. The distance between the pipet tips may be any desired length, approximately $100\ \mu$ is the shortest actually used in the experiments. For testing potential gradients along any particular axis in the brain, it is desirable that the ratio, electrode separation to pipet diameter, be large, in most instances it has had a value of 3 or more. Such micro-electrode pairs may be used in any of several ways. One may record between the two micro-electrodes of such a pair on a single channel, or with two recording circuits or by successive records with one channel, one may record between each of the two micro electrodes and a common, larger electrode ordinarily placed at a distance. It is possible to record reliably certain potentials with an electrode system consist-

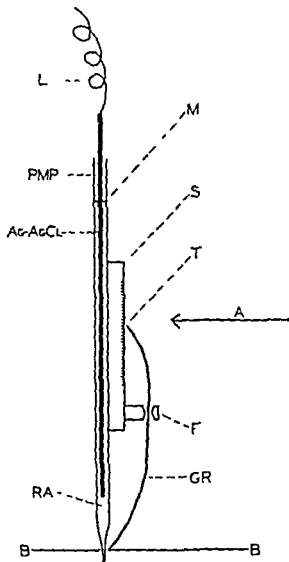


FIG 1. Diagram of a micro-electrode and a device for determining the distance it has penetrated into the brain. *BB*, the surface of a brain into which is inserted the tip of the pyrex micropipet *PMP*. *RA*, Ringer agar filling the micropipet up to the level *M*. *Ag-AgCl*, chlorided silver wire dipping into the pipet. *L*, lead going from the silver wire to the amplifier *S*, a graduated metal scale upon which the micropipet is mounted [The scale is fastened to a rod (not shown) which is carried by the micromanipulator]. *GR*, a fine glass rod which passes through the hole in *F* and rests on the surface of the brain near the point of entry of the micropipet *T*, the tip of the glass rod, its movement relative to the scale is observed with a horizontal microscope the optical axis of which is indicated by the arrow *A*.

* The micropipets have been pulled either by hand or with the machine devised and kindly loaned by Dr L. G. Livingston, from thoroughly clean pyrex capillary tubing (outer diameter, 0.85 mm, inner diameter 0.60 mm).

ing of two high-resistance half-cells, but the difficulties and dangers are much greater than when one of the electrodes is of low impedance. When the amplifying system is not differential it is most important to test whether the potential changes recorded are really occurring between the two micro-electrodes and not between the one connected to the amplifier grid and a diffuse lead on the animal whose circuit to the amplifier ground is completed by capacity and leakage. Such capacitative connections are the more important the higher the frequencies recorded. Tests have proved that potential changes of the duration of the pentobarbital waves of the cortex may be recorded reliably between two micro-electrodes (see below); with much faster changes, of the duration of axon spikes, this is not true. Fortunately, the capacity between the solution and wire within a micropipet and the external conducting medium in which it is placed is not great enough to offer a shunting circuit which appreciably interferes with measurements of even rapid, axon-like potentials; the same is true of the capacity between two closely approximated members of a pair of micro-electrodes.

Effects of the grid current of the first tube of the amplifier flowing through the electrodes must be considered, for because of the very small surface area of a micro-electrode a considerable current density may exist in tissue near its tip due to the passage of only minute currents. Tests showed that the grid current from the amplifier used with the cathode ray tube (see below) flowing through a micro-electrode of tip diameter about $40\ \mu$ had no detectable effects upon axon-like spikes which were being recorded from the hippocampus; the observed activity was unaffected by the presence or absence of a 2-microfarad condenser placed in series with the electrode. Since these are the potentials which arise in the most localized way near the tips of micro-electrodes they are the ones most likely to be influenced by grid current flow.

There are two requirements for the manipulation of micro-electrodes in a controlled way; rigid fixation of the tissue (brain) relative to the electrode, and a method of moving the electrode through the tissue by small steps, as accurately measurable as possible. Satisfactory immobilization of the animal has been obtained by a three-point fixation of the skull. The jaws are fixed with a head-holder of the usual type, arranged to be as rigid as possible, and drills are forced tightly against the mastoid processes of the temporal bones. The electrodes were held and moved by means of the small Emerson micromanipulator. Ordinarily the micropipet is fastened to a small steel scale, graduated in 0.5 mm. intervals, which is carried by a brass rod firmly clamped in the manipulator. A horizontal microscope with ocular scale makes it possible to determine the movement of the electrode relative to its original position with some precision—to $100\ \mu$ or better. The movements of the electrode through the tissue are given less precisely because of the possible compression of the brain; accordingly the actual movement of an electrode relative to the brain may be somewhat more or less than its movement relative to the skull, the manipulator or other fixed points. For this reason a so-called "depth-measurer" has been devised (see Fig. 1). Movement of the electrode scale s , relative to a point on the ocular scale of the horizontal microscope represents the distance which the electrode has been moved vertically. Movement of the end t of the tiny glass rod gr relative to the ocular scale represents the extent to which the brain surface in the region of the penetration of the electrode has been compressed. Movement of t relative to the electrode scale represents the distance to which the electrode has penetrated the brain tissue. The use of such differential measurements is quite essential in investigations of the isocortex. In the case of the hippocampus it is not so necessary if the penetrating electrode is small, for the pial layers are thin and the shape of the tissue is such that it exhibits less tendency to compression.

More precise localization of micro-electrodes requires a histological examination. Fortunately the position of even a small micro-electrode may usually be located in sections because the track of the pipet stands out due to the disruption of the tissue. The histological procedure was patterned after the ones of King (1910) and Sugita (1917, 1918), devised to cause little volume change. The tissue was fixed in Bouin's fluid, cut serially in paraffin at $20\ \mu$ and stained with carbo-thionin. The end of the visible track of injury in the tissue represents the deepest point to which the tip of the electrode has been inserted (Fig. 7B and 7C) and ordinarily may be very precisely located. An intermediate point on the path of the electrode may be marked by a lateral movement of the electrode, carried out with the manipulator, and is without danger of vertical movement if the pipet is sufficiently rigid; the lesion thus produced has a shoulder which may be detected in the sections.

Three types of recording apparatus have been used. In some experiments a direct-

coupled amplifier (described by Forbes and Grass, 1937) was used with a Hindle string galvanometer. The string was generally slightly more than critically damped, the time taken for two-thirds of the total deflection to a constant current was about 6 msec. Two channels of resistance-capacity-coupled amplifiers working into oscillographs writing with pens on a paper tape (as developed by Mr. Albert M. Grass) proved useful when two simultaneous records were desired. The amplifiers could be used either with a grid-ground input connection or differentially. The frequency characteristic was linear to about 80 cycles per second, above which it tapered off gradually. Therefore it was not adequate for recording potentials of brief duration. The grid resistors of the input circuits were only 0.5 M Ω , which led to decreased efficiency in recording with high-resistance electrodes. Using the amplifiers with grid-ground connections to record on two channels between each of two micro electrodes as grids and a common ground, the coupling between the two channels was not significant provided the resistance of the common electrode was very low. Most useful, because of its sensitivity to high frequencies, has been the cathode ray tube with the usual amplifying circuits. The grid resistor was small (0.5 M Ω). The input circuit could be used with differential or grid-ground connections, the grid ground circuit proved satisfactory for leading with one high-resistance and one low-resistance electrode.

The difficult problem of stimulating afferent fibers in the volume of the brain only a few millimeters from the recording electrodes on or in the hippocampus was solved initially by using a thyatron-controlled condenser discharge and a balanced Bishop coil (Bishop, 1927), and later by the use of a circuit suggested by Dr. Tonnes in which thyatron-controlled condenser discharges activate the primary of the General Radio Transformer 578A, the special feature of which is electrostatic shielding between the coils, the secondary leads, shunted by 2000 Ω and each with a 0.005 μ F series condenser, connect with the bipolar stimulating electrodes.

Preliminary experiments were performed on chickens of various ages. Cats and rabbits have been used in most instances, however, either under anesthesia (generally pentobarbital sodium) or decorticate. Decorticate animals were immobilized either by a thoracic spinal section and the cutting of the brachial plexi, or by cervical cord section.

THE ISOCORTEX

Observations

The experience of Derbyshire, Rempel, Forbes and Lambert (1936) suggested that pentobarbital sodium would be a most useful anesthetic for experiments designed to dissect cerebral electrical activity. These authors found that brain-activity patterns under light pentobarbital are similar to the unanesthetized, with predominant excursions of as large as several hundred microvolts occurring at frequencies of 5 to 15 per sec. As the anesthesia is deepened the waves become less frequent (5 to 10 per sec.) and there is a smaller amount of irregular, fast activity superimposed upon them. In very deep stages, waves of the same duration and amplitude may appear as isolated excursions.

Figure 2 demonstrates how greatly the cortical activity occurring under deep pentobarbital differs from the ether pattern (cf. Beecher, McDonough and Forbes, 1938). The electrical changes were recorded between an indifferent ground electrode and a micropipet (diameter 30 μ) which had been lowered about 0.5 mm. into the lateral gyrus (cat). The initial anesthetic was ether (moderately light); this was then discontinued and pentobarbital injected. Under ether rhythmic waves of rather striking regularity at over 40 per sec. characterize much of the record. With the transition to pentobarbital this frequency decreased progressively; at first there was little other change in the characteristic pattern. When the pentobarbital anesthesia be-

came deeper, the isolated excursions mentioned above became strikingly apparent. These unitary-appearing potentials, occurring during the deeper stages of pentobarbital anesthesia, are easier to investigate as isolated events than the small, rapid waves observed with ether.

Using micro-electrodes, it was found that in the deeper stages of pentobarbital the baseline becomes smooth except for occasional isolated excursions which often recur with a remarkable constancy of wave form and seem to have some similarity to the "strychnine spikes" described from the

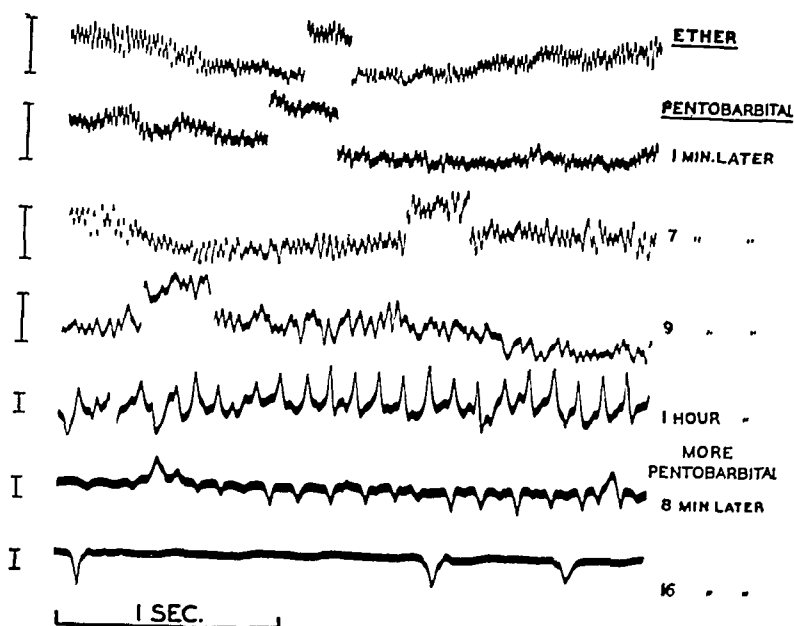


FIG. 2. Records from isocortex of cat; string galvanometer and direct-coupled amplifier. Grid, a $30\ \mu$ micropipet inserted 0.5 mm. into the anterior sigmoid gyrus; ground, a silver plate under the skull. Anesthesia as described on the figure. In each of the first four records a calibration potential of $200\ \mu\text{V}$ was introduced into the circuit for a brief period. $200\ \mu\text{V}$ indicated at left of each record. Upward excursion signifies grid negative. January 25, 1937.

sensory cortex by Dusser de Barenne and McCulloch (1936, 1938). Figures 2 and 4D show characteristic pentobarbital spikes in the cat and Fig. 3 demonstrates that the same phenomena occur in the pallium of the chicken. In the latter, during the deepest stages of narcosis the recurring pairs of excursions, regularly separated by a nearly constant interval, suggest a more or less stable linkage of physiological units.

As recorded in the cat the waves have durations of from 30 to over 150 msec. and may be as large as 200 or even $300\ \mu\text{V}$. These waves may present simple monophasic or diphasic forms or in some cases may be complex. Often a particular wave form may recur at intervals over a considerable period. In moderately deep stages of anesthesia the excursions often appear in series having characteristic rhythms of 5 to 7 per sec. (Fig. 4A and 4B).

The waves of a series sometimes show progressive changes in size (Fig. 4A), suggesting a systematic recruitment of elements. Occasionally the successive waves increase in duration in a way to be expected if activity in component elements were to become more dispersed temporally. Sometimes negative and positive excursions appear in alternation (Fig. 4C). These characteristics justify application of the term "units of electrical activity" to the pentobarbital excursions. It should be pointed out that the nature of the activity under deep pentobarbital may not be due to the drug *per se* but rather to secondary effects referable to its action on blood pressure and possibly other characteristics of the *milieu intérieur* (Beecher, McDonough and Forbes, 1938).



FIG 3 Chicken 3 weeks old, successive stages of pentobarbital anesthesia as recorded with string galvanometer and direct-coupled amplifier. Micro-electrodes (tip diameters 35 and 40 μ) 1 mm apart and pushed just through the surface of the occipital region of the pallium. 200 μ V indicated at upper left. April 27, 1936

The contours of the deflections as shown in string galvanometer records are ordinarily quite smooth, but occasionally show notches or discontinuities which indicate that a more rapid recording instrument might reveal more

jagged outlines indicative of the participation of faster components in the formation of the waves. Furthermore, these excursions have been recorded not only with micro-electrodes but also with macroscopic electrodes on the surface of the brain. There is little doubt, therefore, but that the pentobarbital "units" are functional rather than anatomical and represent activity in a number of cellular elements acting together in an organized way.

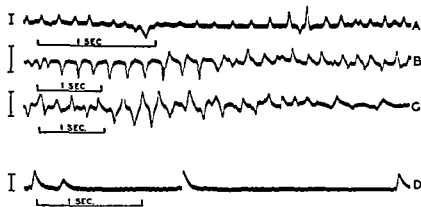


FIG 4 Types of activity in the cerebral cortex of the cat in the deeper stages of pentobarbital anesthesia. Direct-coupled amplifier and string galvanometer. 200 μ V indicated at left. Grnd, a micro-electrode, diffuse ground. April 27, 1936

On the other hand the existence of sharply localized potential gradients, in the vertical axis at least, is suggested by experiments in which the activity recorded between a micro-electrode and an indifferent ground changes greatly as the localizing electrode is pushed into the cortex by small steps. It is the use of pairs of micro-electrodes, however, which has given the most significant information about the spatial relations of activity in the cortex (cat, lateral gyrus) under pentobarbital. The results, based on records taken

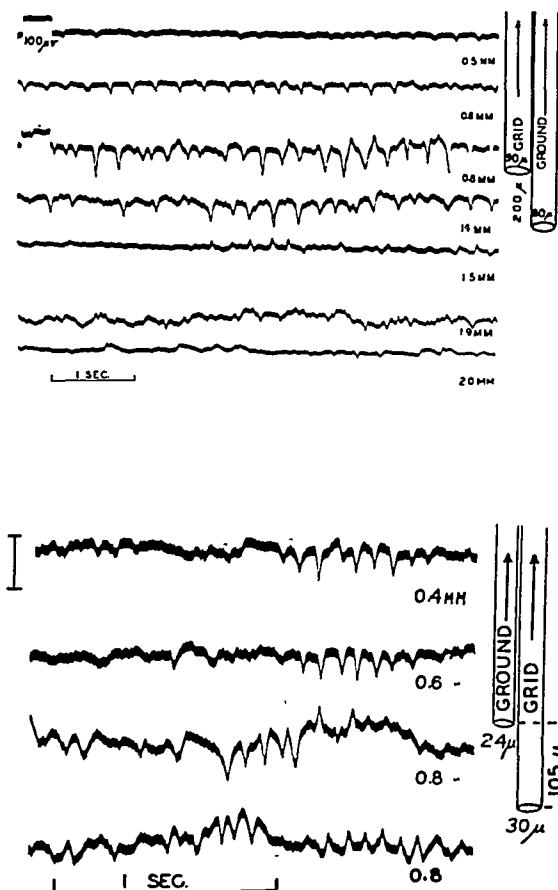


FIG. 5A. Cat under pentobarbital; records from various depths in the lateral gyrus. Electrodes—the microscopic pair shown on the figure. Direct-coupled amplifier and string galvanometer. Upward excursion signifies grid (shorter electrode) negative. Time and voltage scales as indicated. March 5, 1937.

B. Cat under pentobarbital; records from various depths in the lateral gyrus. Electrodes—the microscopic pair shown on the figure. Direct-coupled amplifier and string galvanometer. Upward excursion, grid (deeper electrode) negative. 500 μ V indicated at upper left. March 17, 1937.

activity (Fig. 5A). This fact is proof of localized "pick-up" by this type of electrode pair.

(4) In the lateral gyrus the magnitude of the spikes as recorded from vertical micro-electrode pairs with separation distances as small as 105 μ (the smallest tested) may be as large as 200 μ V (Fig. 5B); no larger po-

with the string galvanometer and the ink-writing oscillographs, may be stated as follows:

(1) Recording from a pair of micro-electrodes with a vertical separation of 105 to 300 μ (the diameter of each pipet being half or less than half of the separation distance), it is found that the activity varies with the depth to which the electrodes have been pushed into the cortex (Fig. 5A). Controls demonstrate that the electrical changes recorded really occur between the two micro-electrodes and not between one of them and a diffuse lead coupled with the amplifier ground by capacity; one could not be sure of this if the potentials were of axon-spike duration or briefer (see page 90).

(2) In the lateral gyrus maximum activity is recorded when the electrodes are at a depth of 0.5 to 1.5 mm. and are therefore in gray matter (Fig. 5A).

(3) Changes of position of the electrodes as slight as 0.1 to 0.2 mm. on the measuring scale (and probably no more in the brain) may cause a drastic change in the recorded electrical ac-

tentials have been obtained regularly from various other combinations of macroscopic and microscopic electrodes placed much farther apart on and in the lateral gyrus.

(5) The potentials between each electrode of a pair and a common gross electrode on the skull recorded simultaneously on two independent channels are sometimes very similar (Fig. 6, records at depths of 0.3 and 1.0 mm.). Under these conditions, the sources of potential change are taken to be at some distance from the micro-electrodes. Localized recording is not indicated.

(6) In some positions of the electrodes, however, the records of two

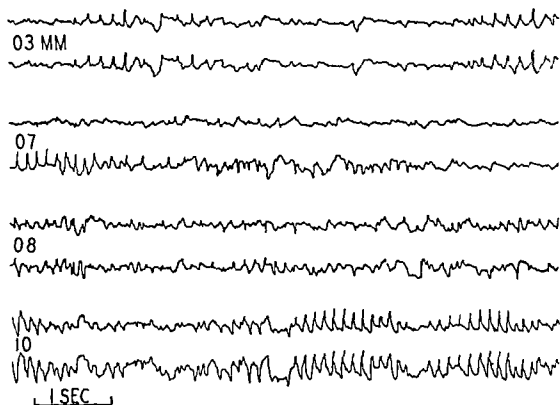


FIG 6 Cat under pentobarbital, tracings of records from 4 positions of different depth in the lateral gyrus. Two-channel recording with capacity-coupled amplifiers and ink-writing oscillographs. Grids, the two electrodes of the pair shown in Fig 5A. Common indifferent ground. Upward excursion signifies grid negative. Time scale shown on the figure. The largest deflections are about 200 μ V. March 17, 1937

channels are much more dissimilar than similar (Fig. 6, at depths of 0.7 and 0.8 mm.). That this condition should prevail at times is a corollary to the fact that large potential differences occur between the electrodes of a microscopic pair (statements 1 and 4). It offers proof both that steep, localized voltage gradients occur in the brain and that micro-electrodes can record these.

Discussion

The data thus verify the two predictions of the potential theory which were mentioned in the Introduction (p. 74); these are that micro-electrodes placed in some positions in active tissue should lead off large potentials and that (corollary to this) under certain conditions they should record in a localized way.

In animals under relatively light chloroform and ether anesthesia, Adrian and Matthews (1934) examined the potentials led from electrodes in various positions on the cortex and in a layer of Ringer's solution placed upon it. The faster components were recorded only by electrodes placed on or very close to the cortex itself and appeared even when the electrodes were only a millimeter or two apart; whereas the slower waves appeared only with electrodes separated by greater distances and were present even if these were raised a few millimeters away from the cortex into the Ringer's solution. Adrian and Matthews concluded that the slow waves (2 or 3 per sec.) were the summations of faster components arising from the somewhat asynchronous activity of a large number of units occupying a considerable cortical area and that the elementary building blocks of which the cortical potentials were formed probably have durations of something like 10 to 100 msec.—relatively brief but still much slower than axon spike potentials.

In the case of potentials led from two micro-electrodes extremely close together in the cortex, the possibilities for spatial summation are greatly reduced because the elements of only a relatively small volume of tissue can contribute significantly to the record. We have not examined cortices under conditions in which slow potential changes form a prominent feature of records taken with gross electrodes some distance apart. The pentobarbital deflections of 30 to 150 msec. duration, however, no doubt represent the faster type of cortical wave. It is therefore in confirmation and extension of the results of Adrian and Matthews that we find this type of activity occurring between points in the cortex separated by only a very small vertical distance.

These results suggest the value of histological preparations making possible correlations between the precise positions (*i.e.*, to distances small compared with 100 to 200 micra) occupied by micro-electrodes and the cellular structures of the region. Observations of this sort have been made, not on the isocortex, but in the hippocampus; further discussion is deferred until the results have been presented.

THE HIPPOCAMPUS

Observations

The hippocampus may be considered as a simplified cortex, for in each of its two principal divisions, the Ammonshorn and the Fascia dentata, one cell layer only is highly developed. Moreover its most numerous cells, the pyramids, are oriented in a relatively simple manner, with the axons, cell bodies and dendrites arranged in fairly well-defined strata. Thus, though the histological structure is of the same general sort as in the ordinary cortex, the geometrical arrangement of the cells and their processes is much less complicated. The presence of afferent pathways which may be stimulated in isolation is extremely useful, for it permits repeated and reproducible synaptic stimulation of the cells of the hippocampus. Further, the finer details of structure have been well worked out, particularly by Cajal (1911) and Lorente de N6 (1934) (see Fig. 7).

The hippocampus is a relatively long body which curves about the floor of the lateral ventricle. Sections perpendicular to the long axis in all parts, except the extreme ends, have nearly the same structure. In the cat and rabbit exposure of the hippocampus by ablation of the isocortex overlying it dorsally reveals that the surface of the part a few millimeters (3 to 8) from the midline is more or less horizontal when the animal's head is in the usual position; this is convenient for the introduction of electrodes. Further, parasagittal sections through the brain in this region are not far from normal to the long axis of the hippocampus. For these reasons this portion was chosen as the most convenient for experimentation.

1. *Injury discharge.* The insertion of an electrode into the hippocampus is often immediately followed by a more or less extensive outburst of electrical changes (Fig. 8 and 9) apparent in recordings taken between this electrode and another placed at a distance. Such activity has been designated "injury discharge" because it is transitory, gradually declining to extinction within a short time—often within 30 seconds. The peak-to-peak voltages generated during such a discharge may amount to a millivolt or more and the form may approximate a sine wave or may consist of approximately monophasic deflections of either polarity or of diphasic deflections with a faster and a slower phase. The slower components of the injury discharge are similar to the "slow waves" to be mentioned below. As recorded with a relatively large electrode (bare silver wire several tenths of a millimeter in diameter inserted for one millimeter into the hippocampus), the faster components rarely have a duration as short as 10 msec.; they are therefore much longer than the axon-like spikes described below. As recorded with a micro-electrode, however, rapid deflections of the duration of axon-spike potentials sometimes, but not always, accompany the slower deflections of an injury discharge; when present the axon-like spikes ordinarily continue long after the slower components have ceased to appear.

As would be expected from mechanical considerations, the injury discharge due to the movement of a micro-electrode in the hippocampus is probably to be related to a disturbance which is limited to a small volume of tissue. The evidence is that, although large potential differences may be recorded between a micro-electrode a millimeter or less below the surface of the hippocampus and a concentric electrode on the surface, corresponding changes on a channel recording between the surface electrode and an indifferent electrode at a distance are small or absent (Fig. 9).

The injury discharge has not been examined in great detail. It is apparent, however, that it resembles in some ways the injury effect reported from the cortex by Adrian and Matthews (1934) and which was relegated by them to the cell bodies and dendrites of gray matter because it was very different from the pattern of small, rapid potentials recorded from the deeper white matter and from nerve trunks pierced by needle electrodes. Bishop (1936) has presented injury discharges from the optic cortex which are not unlike those of the hippocampus.

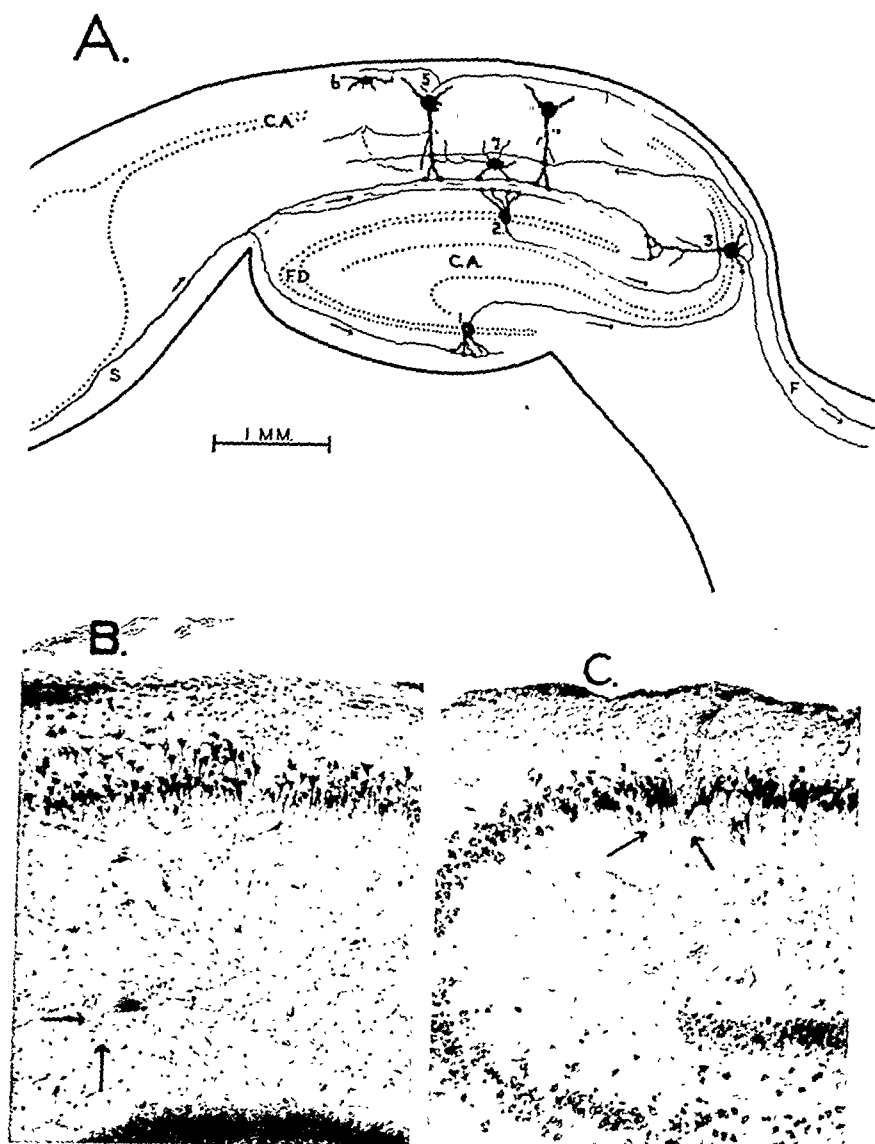


FIG. 7A. A diagrammatic parasagittal section (about 3 mm. from the midline) of the hippocampus of the rabbit. Outlines from an actual section, details from the accounts of Cajal and Lorente de N6. (The size of the cell bodies is exaggerated.) Arrows indicate the direction of transmission of impulses. The hippocampus consists of two divisions, the Ammonshorn (CA) and the Fascia dentata (FD). The main cell type of the Ammonshorn is the pyramidal cell (3, 5); the bodies of these cells form a compact layer (the Stratum pyramidale, CA) which is conspicuous in Nissl preparations (Fig. 7 B and C). The axons of the pyramidal cells are the efferents from the hippocampus; they make up a large portion of the alveus (layer of axons on the dorsal or ventricular surface of the hippocampus) and the fimbria (F). The fimbriae of each side course cephalad and meet; as the fornices they pass ventrad and then caudad to end in the mammillary bodies. The axons of the pyramid basket cells (4) do not leave the hippocampus but form synapses on the cell

2 *Slow waves* A conspicuous type of autonomous activity in the hippocampus as it has been investigated in experiments on cats is what we have termed the "slow wave." In general appearance the slow waves resemble the pentobarbital waves of the isocortex, there are, however, a number of differences

The slow waves are recorded either from gross electrodes on the surface of the hippocampus or from micro electrodes within it. That they are the result of activity of tissue within the hippocampus, and not due to current flow from activity originating in the subjacent thalamus or elsewhere, is indicated by several facts: they may be recorded between two micro electrodes only a few tenths of a millimeter apart in the dorsal part of the hippocampus, two or three millimeters removed from other

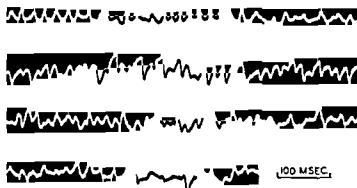


FIG 8 Cat under pentobarbital cathode ray. Records between an indifferent electrode and an uninsulated silver wire, a few tenths of a millimeter in diameter, pushed ca 1 mm into the hippocampus. Records from different positions shortly after inserting the silver wire. No axon like spikes are seen. The larger deflections are about 100 μ V. June 13, 1938

parts of the brain, they are recorded between a micro electrode only 0.2 or 0.3 mm below the surface and a concentric surface lead, as the micropipet is pushed deeper into the hippocampus the sign of the slow waves may change once or even twice, they may be recorded with as high voltage be-

bodies of the pyramids. Some short dendrites of the pyramidal cells pass into the Stratum oriens (subjacent to the alveus), but the most conspicuous ones extend into the hippocampus for several tenths of a millimeter as the long shafts of the Stratum radiatum and arborise terminally in the Stratum moleculare. Afferent fibers (S) from the Area entorhinalis establish collateral synapses with the tips of these dendrites. The principal cell type of the Fascia dentata is the granule cell (1, 2), the bodies of which also form a compact layer (FD). Their dendrites are also in contact with the afferent fibers from the Area entorhinalis. Their axons (the mossy fibers) pass part of the way around the Ammonshorn in two tracts: one on each side of the stratum of pyramidal cell bodies, and make synapses with the dendrites of the pyramidal cells; none of them leave the hippocampus. In addition to the main cell types there are various cells with short axons, of which 6 and 7 are representative. The various regions along the long axis of the hippocampus are correlated by an axial association bundle. Correlation of the various parts of a segment (section perpendicular to the axis) is made possible by recurrent collaterals from the axons of some of the pyramidal cells and by the mossy fibers.

FIG 7B Photograph of a parasagittal section through the hippocampus of a cat, showing a large lesion made by inserting and moving horizontally a pair of micro electrodes. As the movement was made forward (to the right in the picture), the position of the deeper electrode before the horizontal movement is represented by the extreme lower left portion of the lesion indicated by arrows. Nissl stain. November 4, 1937.

FIG 7C Photomicrograph of a parasagittal section through the hippocampus of a cat, showing the lesion made by a pair of micro electrodes pushed into the lower edge of the Stratum pyramidalis. An adjacent section shows a small group of cells immediately below the end of the lesion. Axon like spikes were recorded from this position. Nissl stain. December 23, 1937.

tween electrodes only a small distance (a few tenths of a millimeter) apart on or in the hippocampus as between electrodes much more widely separated but still within the hippocampus.

In some experiments potential changes of this sort may be as large as 200 or 300 μV .; in others they may be inconspicuous or essentially absent. They vary in duration from 20 to 70 msec. The successive excursions on a single record may vary considerably in both magnitude and wavelength. More than that, the wave-form may change from wave to wave, in

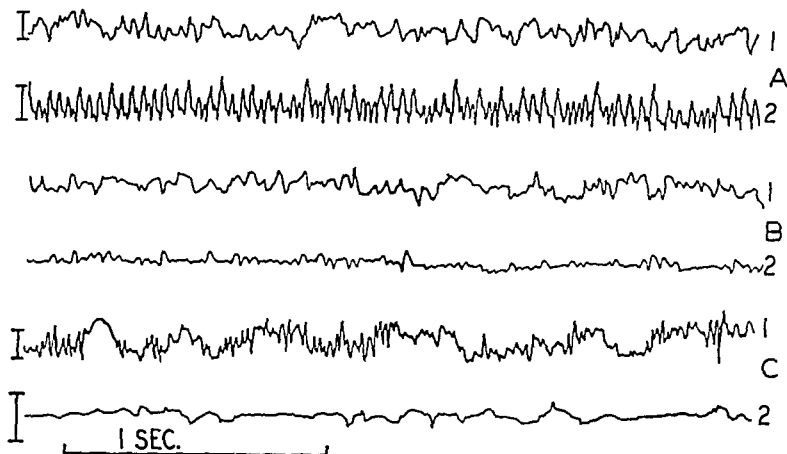


FIG. 9. Cat, hippocampus. Injury discharges recorded with ink-writing oscillographs. Pentobarbital.

A and B, December 4, 1937. Grid of channel 1 an indifferent electrode, grid of channel 2 a microelectrode of 57 μ tip diameter. Common ground for the two channels a silver ring on the surface of the hippocampus, surrounding the micropipet. A, about 20 sec. after moving the pipet from 0.9 to 1.0 mm. into the tissue (depth readings). B, 1 min. later. The baseline of channel 2 later became quite smooth. Note that the large potentials recorded on channel 2 hardly appear on channel 1 in spite of its higher amplification. Voltage calibration for channel 1, 100 μV ; for channel 2, 500 μV .

C, another experiment, December 11, 1937. Grid of channel 1 a micropipet of tip diameter 40 μ . Grid of channel 2 an Ag-AgCl wire on the fimbria. Common ground a semi-circular Ag-AgCl wire about the micropipet on the surface of the hippocampus. Record taken a few seconds after moving the micropipet from a depth of 0.8 to 0.9 mm. (depth readings). The activity of the upper channel rapidly decreased during the next 30 sec. Voltage calibration for both channels, 100 μV . Time as indicated.

that the peaks may be reached after variable fractions of the durations. A most striking feature of the form of the slow waves is that they are usually very nearly or quite monophasic.

In various experiments these waves have been recorded from most of the parts of the hippocampus. As recorded between a localizing electrode in the tissue and an electrically indifferent region, the sign of the slow waves may be either negative or positive with reference to the localizing electrode. It is possible to make certain generalizations relating the position of micro-elec-

trodes in the tissue to the sign of the waves as recorded. Usually during a wave the region of the Stratum pyramidale of the dorsal portion of the Ammonshorn becomes negative to the ventricular surface of the hippocampus or to a remote region. Similarly deeper regions of the hippocampus are often positive and the deepest portions (the region of the lower blade of the Fascia dentata) negative to the surface. Two-channel recording with the two micro-electrodes of a pair as grids and a surface electrode as a common ground indicates that often

two points 0.2 mm., more or less, apart within the hippocampus show similar changes in potential relative to the common electrode; occasionally differences such as those obtained in the isocortex occur. This may be summarized by stating that the mechanisms responsible for the slow waves are not limited to localized regions within the hippocampus and that the volume of tissue active in the production of any particular slow wave is not highly restricted (compare axon-like spikes, described below).

Important for the interpretation of the slow waves is that their contours, even as recorded on the cathode ray oscillograph, show no greater irregularities than

are to be found on other portions of the record—no indications of the participation of fast components in their formation appear. Occasionally an axon-like spike may appear on a slow wave, but it is apparent that this is a chance occurrence and a mere superposition of one type of activity upon another; when the two are present together in a record they appear to be independent. Further, the fast spike is as often superposed on a slow wave of opposite sign as on one of the same sign as itself.

The slow waves generally disappear at depths of anesthesia insufficient to simplify greatly the activity of the isocortex (see above). Though the activity is decreased in amount as the anesthesia is deepened, so that individual waves may stand out more clearly, these do not persist in the very deep stages. In fact at no level of anesthesia do the hippocampal waves ex-

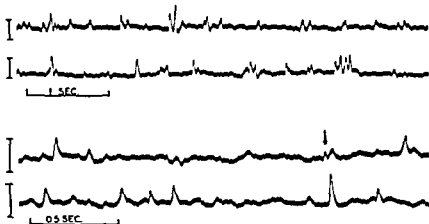


FIG. 10. Cat, hippocampus. "Slow waves." Pentobarbital, string galvanometer and direct-coupled amplifier.

Upper records, experiment of November 5, 1937. A pair of micro-electrodes, one near surface of hippocampus, the other in the Stratum pyramidale (see Fig. 7B). Upward excursion, deep electrode negative. Voltage calibrations, 200 μ V. Time as indicated.

Lower records, experiment of December 14, 1937. Grid, one of a pair of micropipets, the tip in or at the ventral edge of the Stratum pyramidale. Ground, a surface electrode. Note the group of 3 axon-like spikes (indicated by arrow); they had approximately the same amplitude as the slow waves (cathode ray visual observations) but were greatly reduced in this record because of string inertia. In this and all subsequent figures upward deflection signifies grid negative. Voltage calibrations, 200 μ V. Time as indicated.

hibit the regular behavior (repeating wave-forms, rhythmic series of waves) exhibited by the pentobarbital excursions of the isocortex.

3. *Axon-like spikes.* When one of the electrodes of a recording system is

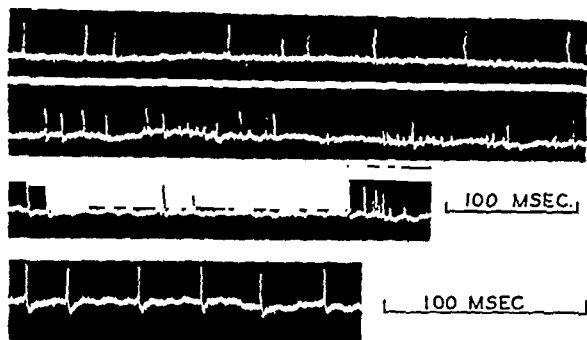


FIG. 11. Cat, hippocampus. Axon-like spikes recorded with cathode ray. Pentobarbital. March 19 and 20, 1938; June 11, 1939. Grid, a micro-electrode within the hippocampus; ground, a macroscopic indifferent lead. In this and all subsequent records upward excursion signifies grid negative. Deflections ca. 500 μ V. Time as indicated.

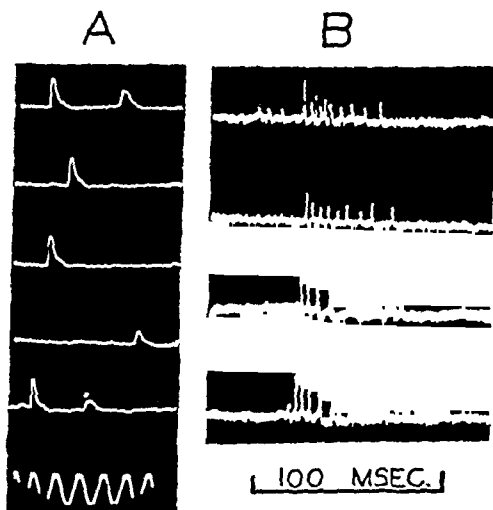


FIG. 12. Cat, hippocampus. Axon-like spikes recorded with cathode ray. Pentobarbital. Electrodes as in Fig. 11. A. High speed record, 500-cycle line at bottom. June 11, 1939. B. Groups of spikes. Time as indicated. March 19, 1938.

* This applies to all spikes of sufficient voltage to rise clearly out of the baseline made noisy by the high resistance of the micro-electrode. Smaller positive spikes may, and we suspect must, occur.

small and is inserted into the hippocampus, rapid deflections often appear. The duration of their conspicuous phase is about 1 msec.; for this reason they have been designated "axon-like spikes." In favorable preparations they may appear as large as 500 μ V; often they are only 150 to 200 μ V. A most constant characteristic is that they are always micro-electrode negative in their predominant phase* (i.e., phase of high voltage), though a smaller and much

longer positive phase sometimes clearly follows the main negative one (Fig. 11 and 12). Frequently the wave-form is simple, but often there may be a hump or notch on either the rising or falling limb of the negativity. This is not always a mere chance overlapping, because what is evidently the discharge of the same unit sometimes appears repeatedly with the same compound wave-form. The occurrence of axon-like spikes ordinarily long outlasts the brief injury discharge. It is true that sometimes they cease to appear a few minutes after a micro-electrode has been moved to a new position, but in other instances they have persisted many minutes and even hours without abatement.

Perhaps the most striking feature of these axon-like spikes is

their frequent grouping in a declining series. It is true that often the spikes appear singly (Fig. 11), and periods of considerable activity may alternate with periods of almost complete inactivity (duration, 30 to 60 sec.). Frequently, however, the spikes come in closely grouped clusters of from 2 to 10 (Fig. 12B). Sometimes the size and spacing of the members of a group appear irregular; examination of the records then suggests that two or more units are involved. In other instances the successive members of a group exhibit a strikingly regular sequence in which they vary progressively and consistently in three ways; they decrease in size, they increase in duration, and the intervals between them increase. The changes in these three characteristics run parallel courses.

A large amount of evidence indicates that the axon-like spikes involve very localized volumes within the hippocampus. They have never been recorded with gross electrodes on or in the hippocampus, but only from micro-electrodes and then only when these were placed in certain, not all, positions within the hippocampus. Even when they appear as large as several hundred microvolts, as recorded between a micro-electrode in the tissue and a concentric surface electrode, they do not appear at all in records taken between the concentric surface electrode and another superficial one placed at a distance. When records are taken of the activity between any surface or distant electrode and a micro-electrode which is lowered into the hippocampus by small steps of about 0.1 mm., axon-like spikes appear in only one or a few vertically localized regions. In several instances paired micro-electrodes with a separation of about 200 μ were lowered into the hippocampus and records taken with these used alternately as grids and a surface electrode as ground. Axon-like spikes appeared first with the lower micro-electrode, then after the pair had been pushed in a little farther they were recorded from the upper electrode, but in no instance from both at a single position. When a micro-electrode which is in a position to record axon-like spikes is pushed in the tissue to a deeper level and then withdrawn to the original locus, the spikes have then never been recorded. It may be concluded that the tissue active in the production of the axon-like spikes is very restricted in volume, that it lies close to the micro-electrode recording the spikes, and that the potential gradients set up by its activity fall off rapidly with distance.

Over 20 depth analyses, most of them controlled histologically, were made to determine where in the hippocampus the axon-like spikes appear. The evidence of these, supplemented by many other observations incidental to other experiments, indicates that the vertically localized regions are within or at least very close to, the Stratum pyramidale—the layer where the cell bodies of the pyramids of the Ammonshorn are located. In a number of instances the spikes have been recorded when the micro-electrode was within the hilus of the Fascia dentata, in Lorente de Nó's area of modified Ammonshorn pyramids, CA4; in other experiments the micro-electrode was within, or in some instances perhaps just ventral to, the Stratum pyramidale

of the dorsal portion of the Ammonshorn. Figure 7C shows the lesion made by a micro-electrode in an experiment in which the axon-like spikes were being recorded. In only two cases do the data indicate that the axon-like spikes appeared when the micro-electrode was in another part of the hippocampus; in one of these the sections showed a lesion well within the Stratum radiatum, in the other the lesion extended to within a very small distance ($50\ \mu$ or less) of the tip of the upper blade of the Fascia dentata, another region of closely packed cell bodies. It is a question whether these exceptions are valid or whether they involve errors in the histological controls.

The systematic investigation of the axon-like spikes was made in the anesthetized cat; they also occur in the decorticate and unanesthetized rabbit. The relation of the axon-like spikes to slow waves was described above; their relation to responses to stimulation will be mentioned below.

4. *Responses of the hippocampus to stimulation of its afferent fibers.* Stimulating electrodes have been inserted into the entorhinal area in order to stimulate the fibers running to the hippocampus (S, Fig. 7A). Ordinarily, one recording electrode was placed on the hippocampus and another put at some other position on or near the brain. Up to several per second, the frequency of stimulation had little effect on the response; stimuli were given therefore at rates of 1 to 3 per sec.

Typical responses taken from several experiments are shown in Fig. 13, A-R. In each of the experiments, activity was detected over a large area of the surface of the hippocampus; the details but not the general aspects of the responses varied with the position of the hippocampal electrode. The position of the other recording electrode was shown to be immaterial. In both cats and rabbits the first phase of a response typically represents positivity at the surface of the hippocampus. It has a latency of about 1 to 4 msec. and a duration of 6 to 20 or more msec.; it may be as large as 1 mV. in the cat under pentobarbital and much larger in the decorticate rabbit. With weaker stimuli the positive phase is generally simple; as the stimuli are increased in strength the responses become larger and sometimes more complex; often they assume a dicrotic shape. The positive phases of the responses to a slow series of uniform stimuli are very constant. The positive phase is often followed by a negative deflection which exhibits considerable variation in its size and duration, even within a single series of uniform stimuli. In some experiments it is not detectable; in other instances it is larger than the positive phase. Its duration, measured from the stimulus, varies from 40 to over 100 msec.

We have not yet obtained records from the fimbria or fornix which we can definitely state represent activity in the axons of this efferent pathway, uncontaminated by the response of the hippocampus itself. Figure 13J demonstrates that the fibers of the fimbria were conducting, however. In this case the stimulating electrodes were on the fimbria several mm. from the hippocampus and the active recording electrode was on the anterior part of the hippocampus. The di- or perhaps triphasically recorded spike repre-

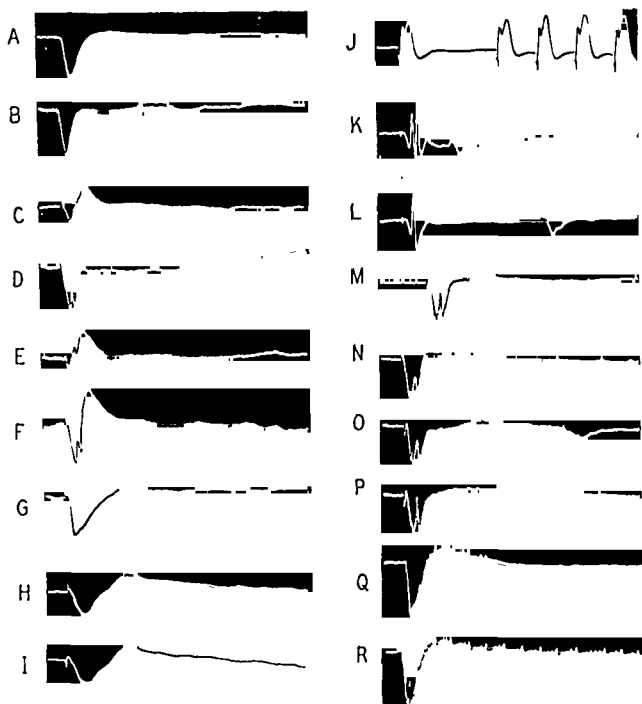


FIG. 13. Hippocampus. A-F, J, M-P, cats under pentobarbital; G-I, K, L, Q, R, decorticate rabbits. A-I, various forms of the responses to stimulation of afferent fibers from Area entorhinalis. Grid, a macroscopic electrode on the hippocampus; ground, an indifferent electrode. I, same as H, but apparatus with a much longer time constant than usual to demonstrate that the negative phase is little distorted with the customary coupling. J, stimulation of the fimbria; monopolar recording from the anterior border of the surface of the hippocampus. K-L, same as A-I, showing spike complication. M-P, responses to stimulation of Area entorhinalis, recorded from micro-electrode in hilus of Fascia dentata. Q, R, responses recorded from a micro-electrode ($30\ \mu$ tip diameter) inserted into the Area entorhinalis close to the stimulating electrodes. Duration of each record, 155 msec.

senting impulses in the axons of the fimbria is followed by the larger and slower potential changes referable to activity in the hippocampus. Responses of the hippocampus to stimulation of the entorhinal area are often complicated by what is apparently the superposition of one or two spikes on the positive potential (Fig. 13, K, L; see also Fig. 16B). These are often large

and have a duration of 2 to 3 msec. They are recorded both from surface leads and from electrodes placed within the hippocampus. What may be the components of such spikes have been recorded on a few occasions from micro-electrodes placed within the hippocampus. Fig. 13, M-P shows four responses taken from a micro-electrode placed within the hilus of the Fascia dentata. The response in this position happened to have been recorded with much the same form as the surface response, but during the period of the positive potential there appeared very rapid spikes which vary con-

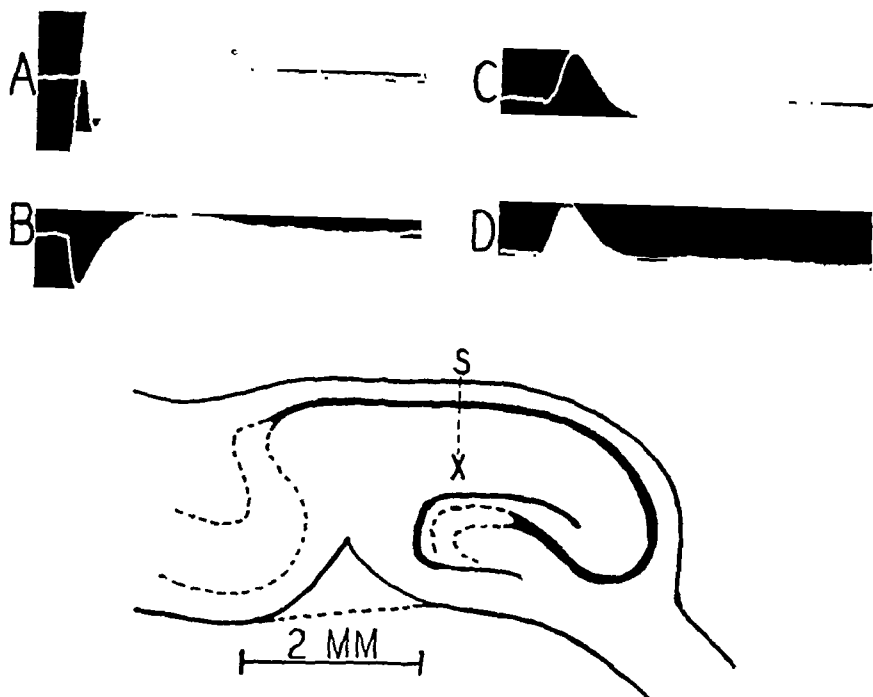


FIG. 14. Hippocampus, decorticate rabbit. Monopolar recording from micropipet of *ca.* $30\ \mu$ tip diameter. Stimulation of Area entorhinalis. A, B, micro-electrode on surface at point S. C, D, micro-electrode inserted into position X. Duration of each record, 120 msec. Responses *ca.* $700\ \mu\text{V}$. Simultaneous ordinates shown on A. Diagram traced from a section showing the lesion made by the micro-electrode. July 29, 1938.

siderably from record to record. Their principal phase seems to be negative—opposite in sign to the response on which they appear to be superposed. Spontaneous axon-like spikes were recorded from this position of the micro-electrode. Fig. 13 Q, R shows some responses taken with a micro-electrode inserted not into the hippocampus but into the entorhinal area near the stimulating electrodes. In this experiment spikes were superposed on the responses and continued in some cases as a short after-discharge; a series of stimuli at *circa* 50 per sec. was followed by an after-discharge of these rapid spikes lasting several seconds and superposed during its earlier part on slow deflections of the baseline. These spike complications do not appear in all

experiments, and when present they seem to be superposed on the slower and universally present components of the response. Consequently our analysis has been concerned only with the slower components and the interpretation of the spikes must await further experimentation.

Micro-electrodes have been used to examine the distribution of the potential changes which occur within the hippocampus during the responses to stimulation. Significant reversals of sign of electric response appear at certain depths. The rabbit has proved much more satisfactory than the cat for this purpose, both because the voltage of the responses is greater and because the hippocampus and related structures have a more favorable topography, being flatter and more spread out. Not all of the experiments are readily subject to analysis, but when certain conditions prevail it is possible, in our opinion, to relegate the greater part of the recorded responses to the perikarya of the pyramids of the Ammonshorn. These conditions are (1) that the responses as recorded from the surface of the hippocampus be approximately the same over a large area, on the central portion of which the micro-electrode is placed and inserted; (2) that the responses as recorded from this area be relatively large compared to potentials recorded from other regions such as the Area entorhinalis in the vicinity of the stimulating electrodes; and (3) that the major potential gradients set up within the hippocampus occur in the dorsal portion of the Ammonshorn and not in the deeper parts of the hippocampus.

That the third condition is not always true is demonstrated by the records of Fig. 15A, which represent the responses as recorded between an electrode on the surface of the hippocampus and a micro-electrode inserted to various depths within the tissue. At the end of the experiment the electrode at a depth of 1.5 mm. was moved laterally to produce a shoulder on the lesion; the sections demonstrated that this position corresponded to about the level to which the tips of the dendrites of the pyramids of the upper portion of the Ammonshorn extended ventrally. It is seen that a conspicuous change in the recorded activity occurred below this level. In other instances records taken between a micro-electrode and a remote, indifferent electrode demonstrate that the response with the micro-electrode in the deeper portions of the upper part of the Ammonshorn is roughly of the same wave-form and voltage as the surface response but of opposite sign, and conspicuous changes in potential do not occur at deeper levels (Fig. 14, 15B, and 16A). In experiments giving this result, records taken at a number of intermediate vertical positions demonstrate that the level at which the sign of the response reverses differs according to what portion of the response is examined; but it is also apparent that this band corresponds to the more ventral regions of the Stratum radiatum, and not to the region of the Stratum pyramidale. Fig. 15B shows an experiment in which the response appears to consist of at least two components which reverse at somewhat different depths; in most experiments such a clear separation of components in the response does not occur.

Fig. 16B shows records from one experiment in which the responses were most significantly atypical. That the excitation of the hippocampus was different from the usual is indicated by the facts that the latency of the

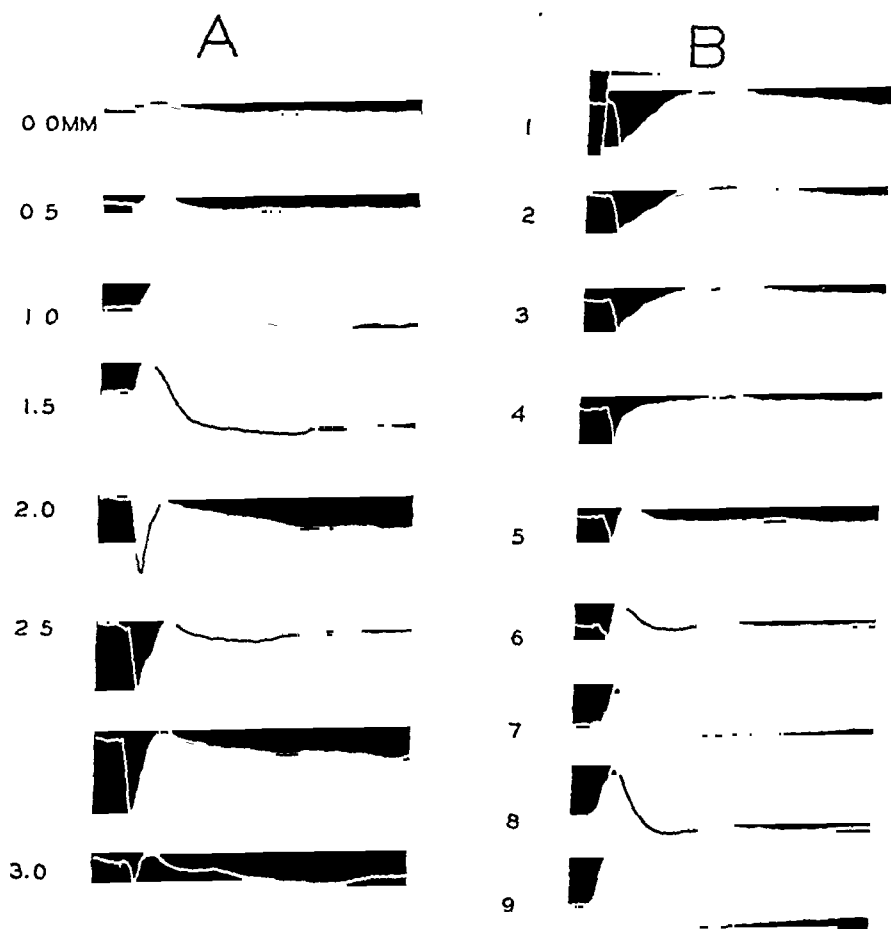


FIG. 15. Hippocampus, decorticate rabbit. Stimulation of Area entorhinalis. Duration of each record about 130 msec.

A. Recording from a micro-electrode at various depths in the hippocampus and an adjacent surface electrode. Slides showed that the depth reading of 1.5 mm. was at about the ventral border of the dorsal portion of the Ammonshorn. Sept. 23, 1938.

B. Monopolar recording from a micropipet at various depths in the hippocampus, from the surface (1) to a depth reading 1.3 mm. (9), which is about the ventral border of the dorsal portion of the Ammonshorn. Simultaneous ordinates shown on (1). August 8, 1938.

principal response (which was very large—ca. 7 mV), instead of being one or a few milliseconds, was about 20 msec. and that the sign of the response was surface negative in contrast to the typical surface positive. The micro-electrode was inserted, and it was discovered that the sign of the response

reversed at the shallow depth of 0.3 to 0.5 mm., in the region of the cell bodies and proximal portions of the dendrites of the pyramids. It should be emphasized that although the results obtained in this experiment were exceptional, a large number of records over a considerable period of time established their constancy for this particular preparation.

Discussion

A sharp distinction may be drawn between the axon-like spikes and the slow waves. Although we do not know what the slow waves signify in terms of the activity of neurons, no evidence has yet appeared to indicate that the latter are the summation products of numbers of axon-like spikes or other rapid components. No intermediate forms are seen. Though they have 10 to 100 times the duration of the spikes, the slow waves have contours which are smooth to the limit of resolution of the recording system. When the two types of activity occur together in the same record they show no interdependence; neither seems affected by the other even when, as occasionally happens, they are superposed. One type of activity may occur without the other. Slow waves may be recorded from macroscopic electrodes on the surface of the hippocampus; axon-like spikes never have been, in spite of their equally great or even greater voltage as recorded with micro-electrodes. Recording between an electrode on the surface of the hippocampus and a micro-electrode within it, the slow waves may be detected when the micro-electrode is in almost any position and the deflections may be either micro-electrode negative or positive; the axon-like activity, on the other hand, is obtained only from certain restricted portions of the hippocampus and is always micro-electrode negative in its conspicuous phase.

What are the axon-like spikes? Their characteristics as enumerated above—in particular, their brief duration, the restricted volume of tissue from which they are recorded, and their systematic behavior in the groups which appear in some experiments—suggest that each must represent activity in a single neuron or in a small group activated by a common agent (compare motor unit of Sherrington). It is possible that they are injury effects (cf. Adrian, 1930) due to the presence of the micro-electrode which is instrumental in recording them—since they are only recorded from micro-electrodes placed within the hippocampus, this point cannot be settled directly. A propagated disturbance arising in a portion of a neuron near the micro-electrode and traveling away to more remote portions of the same cell would account for the prominent initial negative phase and the smaller but more prolonged positivity which is seen to follow in some records (Fig. 11). On the other hand, the spikes are apparently associated with the Stratum pyramidale. In some instances spike-like activity follows the stimulation of afferent tracts to the hippocampus, appearing either superposed on the responses or as an after-discharge (Fig. 13, K-R). Furthermore, the occurrence of the spikes in groups (Fig. 12B) parallels the activity, shown not to

be an injury discharge, recorded by Adrian and Moruzzi (1939) in the fibers of the pyramidal tracts from the cortex. This evidence is not of a conclusive nature, but it leaves open the possibility that the axon-like spikes represent physiological activity in which the bodies of pyramidal cells play an essential rôle. The simultaneous decline of voltage, brevity and frequency in the groups of axon-like spikes suggests the development of fatigue or subnormality in units rhythmically or continuously stimulated, and consequent cessation of discharge.

The examination of the axon-like spikes, and particularly the results of experiments with two micro-electrodes close together in the hippocampus, has afforded convincing evidence of (1) the localized recording which micro-electrodes permit and (2) the restricted volumes within which the electrical changes due to activity in single units are large enough to be recorded readily.

Generally during the first part of the responses of the hippocampus to the electrical stimulation of afferent fibers the surface is positive to the deeper parts of the dorsal portion of the Ammonshorn; but in the exceptional experiment (Fig. 16B) the reverse is true. Thus the difference in potential which arises across this cell layer due to activity of the hippocampus may be either of one sign or the other.

If we may interpret voltage changes such as we have recorded in terms of potential differences originating at the surfaces of neurons, then as was pointed out above, there is required the production of a greater change in potential in some portions of the active neurons than in other parts of the same cells.* The diphasic recording of axon spike potentials in nerve trunks fulfills this condition. But the length of the region active during the passage of a propagated disturbance in nerve fibers is great compared to the entire extent of many cells of short axon; it is as much as several centimeters in mammalian A fibers (Gasser and Grundfest, 1936) and a few millimeters in mammalian C fibers (Grundfest and Gasser, 1938). Consequently, though little is known of the nature of processes occurring in perikarya, it is likely that the production of the large potential differences in the gray matter is due less to the movement of a region of altered potential in the neuron than to the approximately simultaneous production of different changes of potential in adjacent regions (cf. Hughes and Gasser, 1934; Adrian, 1936). For each point on the surface of a neuron a curve might hypothetically be constructed to relate potential change with time. If during activity of the neuron discrepancies exist between the curves for the points on one portion of the neuron and for those on another, then current must flow from the one portion to the other in the medium bathing the cell. Con-

* Because in the present work only transient changes of a duration of no more than 200 msec. are considered, it makes little difference whether the entire surface of the inactive but living neuron is isopotential (as is commonly assumed) or not (cf. Buchthal, 1937, for the case of the region of the motor end-plate); we are concerned only with changes from the resting state.

ceivably such differences may exist between the axon and the perikaryon as a whole, or between different parts of the perikaryon. When present they may properly be called cell potentials and be measured in terms of the voltage drops (cf. Dusser de Barenne and McCulloch, 1939) occurring in the medium bathing the cells.

In some experiments at least the greater part of each of the responses recorded from the surface of the hippocampus and from within the Ammonshorn must be relegated to elements oriented vertically in the Ammonshorn. The potential changes are large (1 to several millivolts in the rabbit). They appear simultaneously and of approximately the same voltage and wave form over a considerable area of the surface. On the other hand, marked changes occur in the vertical direction, and these (in some experiments) are more pronounced in the Ammonshorn than in deeper regions of the hippocampus. The responses within the deeper portions of the (dorsal blade of the) Ammonshorn are reversed in sign but of approximately the same voltage and wave form as the surface response. Furthermore, the changes in the vertical axis are apparently not mirrored in any particular horizontal plane or planes, as might be expected if they arose in horizontally oriented elements. In the Ammonshorn there are, of course, many axons coursing in various directions, particularly in horizontal planes. Cells of short axon are also present in the Strata oriens, radiatum and moleculare, but in very restricted numbers (Fig. 7B and C). The pyramidal cells, however, comprise by far the most numerous cellular elements of this region, and they are vertically oriented. Consequently we infer that the greater part of each of the recorded responses is due to activity of the pyramids.

Just how closely the recorded responses parallel the sequence of changes in a single neuron is not clear. Probably the pyramidal cells are not activated merely by a single volley delivered to the synapses at the tips of the dendrites by fibers from the Area entorhinalis. The stimulus may activate the Area entorhinalis to a more or less extended discharge. Though prolonged reverberation within the hippocampus is not likely, delayed impulses presumably reach the Ammonshorn pyramids from the axons of the granule cells of the Fascia dentata, from the occasional cells of short axon in the Ammonshorn, and from the powerful recurrent collaterals of the pyramids themselves. Furthermore, there is the possibility of reverberation in longer circuits involving both entorhinal areas and both hippocampi (cf. Cajal, 1911; Lorente de Nó, 1934). Unfortunately also the character of the discharge from the pyramidal cells—the activity in the axons of the fimbria—is not yet clearly understood. Figure 13J shows that these axons could be excited directly in our experiments. It also shows that when this is done the spike potentials of even a big synchronous volley are somewhat dwarfed by the large responses apparently arising in the Ammonshorn. The spikes are recorded di- or tri-phasically, so that the voltage change to be recorded as a result of even a considerable amount of somewhat dispersed activity must be small. The short length of the axons and their relation to the hippo-

campus with its large responses has prevented us from determining with certainty what efferent impulses are associated with activity in the Ammons-horn. The "spike complex" shown in Fig. 13, K, L, and Fig. 16B may be associated with efferent discharge. However that may be, the general form of the typical response recorded from the hippocampus conforms to a pat-

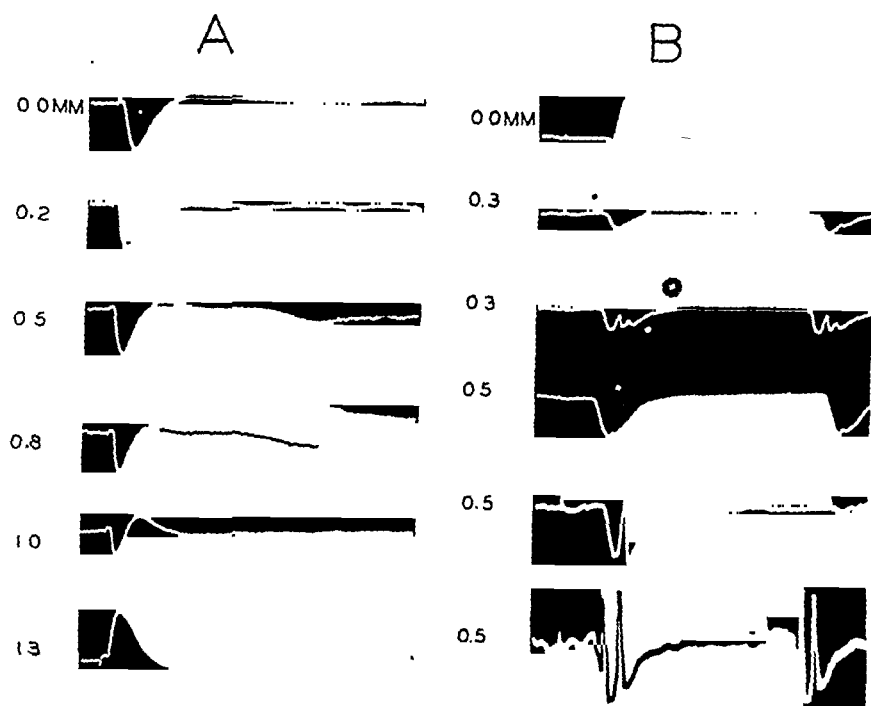


FIG. 16. Hippocampus, decorticate rabbit. Stimulation of Area entorhinalis. Monopolar recording from micropipet (tip diameter ca. 25μ) at various depths in the hippocampus.

A. A typical experiment; a surface-positive response of short latency, reversing in sign in the deeper parts of the dorsal portion of the Ammons-horn. Responses about 1 mV. Calibration on record at 0.8 mm. depth reading. Duration of records, 155 msec. Simultaneous ordinates as in Fig. 15 B. August 8, 1938.

B. The atypical experiment; a surface negative response of long latency, reversing in sign at 0.3 to 0.5 mm. depth reading, at about the level of the Stratum pyramidale. The facilitating effect of a fairly rapid series of stimuli was required to obtain these large responses (7 mV). The last two records were obtained later than the previous ones and with stronger stimuli. Duration of records, 160 msec. September, 22, 1938.

tern found in other parts of the nervous system (cf. Erlanger and Gasser, 1937; Eccles, 1936; Granit, 1933; Gasser and Graham, 1933).

Typically the response of the hippocampus when uncomplicated by spikes is surface positive and reverses in sign as a micro-electrode is pushed into the deeper portions of the Ammons-horn, so that the zone of reversal of potential—the vertical region whose potential is equal to that of an indifferent point—lies in the region of the deeper portions of the long spike

dendrites of the pyramids. In so far as the potential differences are due to the pyramidal cells, this signifies that the majority of active neurons have become relatively depolarized below this level—at their dendrite tips—and less so (if at all) in their more dorsal regions. It is true that activity in all units in the remainder of the hippocampus and even in more distant regions contributes, theoretically at least, to the potential changes occurring at points within the Ammonshorn. The large size and the rapidity of reversal of the potential changes in the Ammonshorn makes it fairly certain that such effects are not great enough to alter the conclusion that the pyramidal cells become, due to their own activity, relatively negative in the deepest portions of the dendrites and positive (relative to a point whose potential does not alter) in the cell body and the proximal portions of the spike dendrites.

Any complicating effect due to activity elsewhere must be even less in the atypical experiment (Fig. 16B), for not only were the potentials extremely large but the reversal of sign of the response occurred at a much shallower level, further from other elements outside the Ammonshorn. In this experiment the dorsal portions of the majority of active pyramids became negative to the deeper parts. The precise level of reversal was not clear, but it was certainly close to the cell body, so that in the majority of active cells the region of the axon and the dendrites in the Stratum oriens must have been negative relative to the spike dendrite extending ventrally.

Adrian (1936, 1938) has recorded potential changes from the surface of the electrically stimulated cortex, which resemble the hippocampal responses. He showed that, in some cases at least, the potential changes which he termed the "deep response" are due to activity in the vertically oriented pyramidal cells. In these responses the surface of the cortex became electrically positive to an indifferent region. He interpreted this as indicating that the deeper portions (part of the cell body) of the pyramids become more depolarized than the apical dendrites which extend toward the surface of the cortex. Since the orientation of the cells of the Ammonshorn is the reverse of that in the cortex, the distribution of potentials in our atypical experiment is similar to that found in the isocortex by Adrian. Dusser de Barenne and McCulloch (1938) have examined cortical activity by the use of a combination of three analytical methods—strychnine poisoning, thermo-coagulation, and depth analysis with micro-electrodes. They found that the "strychnine spike" may be analyzed into an earlier component represented by negativity (relative to an indifferent electrode) in the deeper layers of the cortex and positivity at the surface; a later component whose sign is the reverse of the first; and a final phase which is presumably similar to the first. It seems that a possible explanation of these results would be in terms either of a shifting locus of relative negativity in a single group of vertically oriented elements (pyramids) or, more probably, of the development of a locus (which shifts less pronouncedly) in each of two groups of elements. Lorente de N6 (1939) has recently obtained evidence that after

campus with its large responses has prevented us from determining with certainty what efferent impulses are associated with activity in the Ammonshorn. The "spike complex" shown in Fig. 13, K, L, and Fig. 16B may be associated with efferent discharge. However that may be, the general form of the typical response recorded from the hippocampus conforms to a pat-

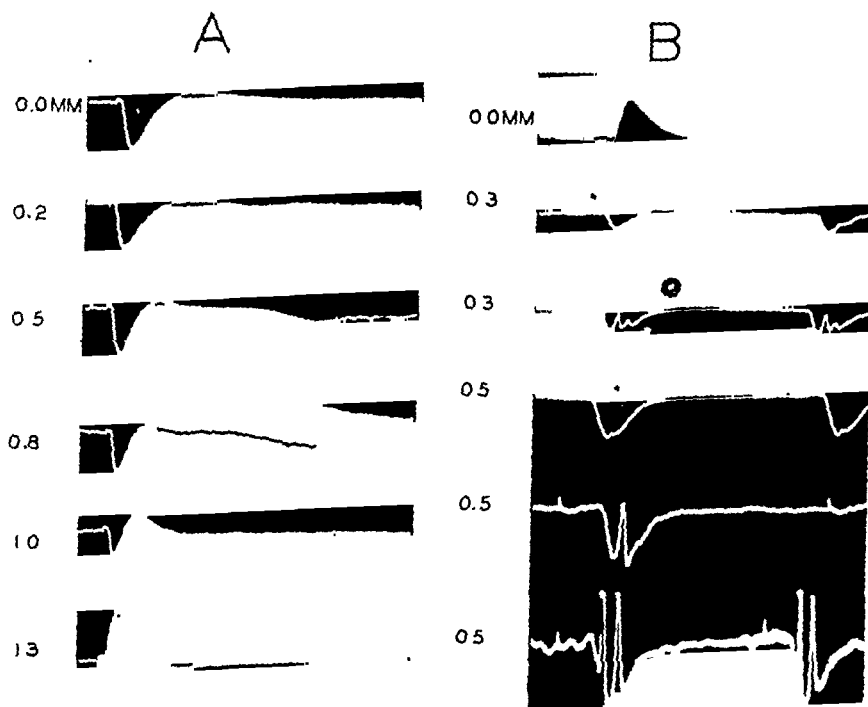


FIG. 16. Hippocampus, decorticate rabbit. Stimulation of Area entorhinalis. Monopolar recording from micropipet (tip diameter ca. 25μ) at various depths in the hippocampus.

A. A typical experiment; a surface-positive response of short latency, reversing in sign in the deeper parts of the dorsal portion of the Ammonshorn. Responses about 1 mV. Calibration on record at 0.8 mm. depth reading. Duration of records, 155 msec. Simultaneous ordinates as in Fig. 15 B. August 8, 1938.

B. The atypical experiment; a surface-negative response of long latency, reversing in sign at 0.3 to 0.5 mm. depth reading, at about the level of the Stratum pyramidale. The facilitating effect of a fairly rapid series of stimuli was required to obtain these large responses (7 mV). The last two records were obtained later than the previous ones and with stronger stimuli. Duration of records, 160 msec. September, 22, 1938.

tern found in other parts of the nervous system (cf. Erlanger and Gasser, 1937; Eccles, 1936; Granit, 1933; Gasser and Graham, 1933).

Typically the response of the hippocampus when uncomplicated by spikes is surface positive and reverses in sign as a micro-electrode is pushed into the deeper portions of the Ammonshorn, so that the zone of reversal of potential—the vertical region whose potential is equal to that of an indifferent point—lies in the region of the deeper portions of the long spike

considerable size and duration appear; their characteristics are such that they must be due to organized activity in groups of related neurons.

4. Further experiments have been performed on the hippocampus (cat; rabbit) because of its relatively simple structure. In addition to changes manifestly due to stimulation by injury three types of activity may be described.

5. "Slow waves" appear as spontaneous excursions of 20-70 msec. duration. They are recorded from the surface of the hippocampus as well as from micro-electrodes placed at various positions within it. Their nature is obscure but there is no evidence that they are composed of overlapping spike-like components.

6. Rapid deflections of about one millisecond in duration and always negative in their predominant phase are recorded only from micro-electrodes placed in or very near the strata containing the cell bodies of the pyramidal cells of the Ammonshorn.

7. The stimulation of the afferent fibers going to the hippocampus from the Area entorhinalis results in responses which may be recorded from the surface of the hippocampus and from points within it. The surface response ordinarily appears after a latency of one or a few milliseconds and is characterized by an initial surface positive phase (10-20 msec.) followed by a smaller, longer and more variable surface negative phase. The responses recorded from micro-electrodes inserted within the hippocampus appear of opposite sign when the micro-electrode (monopolar lead) is in the deeper parts of the Ammonshorn. In a significant atypical experiment the response was surface negative and reversed at a shallower depth in the hippocampus. The results have been analyzed in terms of potential theory and the membrane hypothesis. It is concluded that the responses to stimulation are due largely to potential changes characterizing the activity of the perikarya of the pyramidal cells of the Ammonshorn.

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FACILITATION AND DIFFICILITATION EFFECTED BY NERVE IMPULSES IN PERIPHERAL FIBERS

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SUMMATION and inhibition of impulses in the nervous system must occur at points where they are transferred from one responding unit to another, since an impulse initiated in any part of a normal neuron inevitably traverses the whole of it. Therefore, in attempting to ascertain whether these reactions can occur in nerve fibers an artificial synapse or physiological discontinuity must be provided. Such a synaptic relation cannot be effected by the approximation of two medullated fibers since it has not been possible to demonstrate a measurable effect on one medullated fiber of activity in a contiguous fiber (Blair and Erlanger, 1932), though this does seem to be possible in the case of nonmedullated fibers (Jasper and Monnier, 1938). Neither can a valid artificial synapse be made by damaging a fiber locally since, as is well known, any disruption of the fiber's structure lowers its electrical resistance locally and so reduces the number of current lines eddying ahead from active fiber across the damaged locus, to the fiber to be activated beyond.

In anode polarization, however, one has a means of producing at a node of Ranvier a local discontinuity without changing the structure or lowering the local ohmic resistance of the fiber and thus can interpolate an artificial discontinuity which presumably resembles the natural synapse in some of its electrical aspects, at least. Through the use of this method it has been possible to demonstrate the transmission of a nerve impulse across a non-responding gap in a fiber (Blair and Erlanger, 1939), the temporal summation of two nerve impulses (Blair and Erlanger, 1936), and that (Erlanger, 1939) such summation has the temporal characteristics of the summation of two nerve impulses at the neuromuscular junction, as described by Bremer and Homès (1930). Moreover, casual reference has been made to observations on the temporal summation (facilitation) by multiple impulses in such a preparation (Erlanger, 1939).

The present paper concerns itself with the latter set of observations, which includes an attempt to determine the magnitude of facilitation as affected by the number and by the spacing of the blocked spikes. It deals also with the consideration of a process, designated *difficilitation*,* characterized by an increase in the degree of block, and elicitable by a conditioning tetanus followed by a pause. In addition, evidence, not yet conclusive, will be presented indicating that the action potentials so blocked may be directly responsible for the continuance of this state of *difficilitation*. Such *difficilitation* then would be comparable with inhibition.

* Substantive coined from *difficilitate*, the antonym of *facilitate*.

METHODS

Action potentials of single nerve fibers of the phalangeal preparation of *Rana pipiens* have been observed or recorded with the electron oscillograph by techniques previously described (Blair and Erlanger, 1933; Erlanger and Blair, 1934). The stimulating electrodes are on the central end of the preparation. The anode of a polarizing circuit is connected with the proximal lead electrode and the cathode rests on the nerve a few millimeters central to it, both on the peripheral end of the preparation. The voltage needed to produce at the anode a conduction discontinuity of the desired grade is read on a voltage divider. In long-continued experiments block sometimes develops at the cathode; it can be recognized readily by the failure of that fraction of the conducted action potential to appear in the record seen when the block is at the lead (the anode). The polarizing electrodes are calomel half-cells; all other electrodes are of silver.

The stimuli consisted of trains of shocks delivered through the device diagrammed in Fig. 1. From it could be obtained (i) single trains consisting of any desired number of shocks at any rate and strength, and (ii) two such trains spaced by any desired interval. When the rotor-operated keys were employed the longest cycle available had a duration of about two seconds. For long tetani or wide spacings between them the rotor was disconnected and the bursts controlled manually, but the manual method could be used satisfactorily only when the experiment did not require accurate control of the timing of the bursts or of the intervals between them. The amplifier was of the capacity-coupled type; therefore slow alterations in the level of the fluorescent spot sometimes seen in the records are without significance; some of them were extraneous in origin.

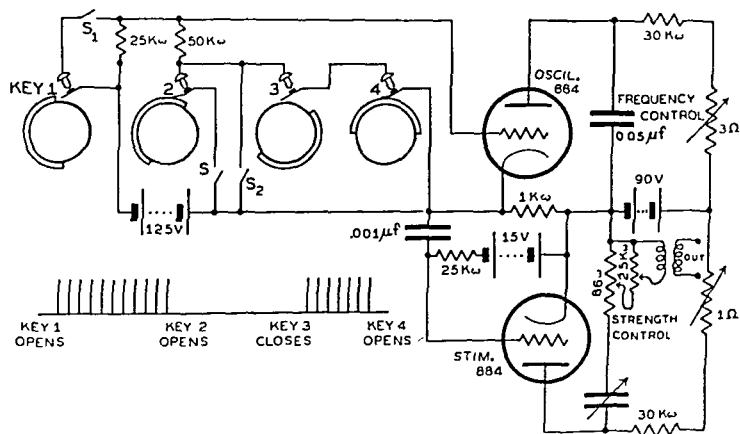


FIG. 1. Diagram of variable frequency stimulator delivering two bursts separated by a variable interval. Simultaneous opening of S_1 and closing of S_2 (D.P.D.T. switch) permits of manual control of stimulation through the master switch, S . The lower diagram shows what operation of the keys accomplishes, the verticals here representing stimuli. The rotating discs are a part of the rotor for synchronizing sweeps and stimulations. $K\omega = 1000$ ohms. $\Omega = 1$ megohm.

The preparations were stored in Ringer solution at 5°C. and, when mounted for study, were dripped with Ringer solution at room temperature. The Ringer solution was made according to the formula given by Bayliss (1920). Its pH was 8.04.* Some nerves failed to exhibit the phenomena under investigation; then it usually was possible to elicit them by increasing to three times that specified by Bayliss the calcium content of the solution applied to the phalangeal end of the preparation.

In this series of experiments much more difficulty than heretofore was experienced in limiting the response at the lead to that of a single fiber, due to the fact that a stimulus which, when applied singly, just reaches the threshold of the fiber of outstanding excita-

* Determined by Dr. H. B. Peugnet.

bility, is apt when rapidly repeated, to attain by temporal summation the threshold of other of the less irritable fibers extending the length of the preparation, an effect Gasser (1938) has compared with recruitment. However, we have through the months succeeded in making many preparations in which but one fiber has conducted to the lead throughout a set of tests. But when the responses of not more than two or three fibers are brought into evidence by recruitment, and their spikes are distinguishable by differences in configuration such as are determined by differences in height or diphasicity, it still may be possible to interpret the pictures in terms of facilitation or of difficilication, as will become evident.

RESULTS

Facilitation. Some conditions affecting the measurement of facilitation. As a measure of the transmissibility of nerve impulses across an anode block we have used the degree of polarization required to maintain the block. The polarizing current is adjusted by means of the voltage divider so that it just suffices to block the desired number of the impulses of a tetanus, the first, for example, the first two only, and so on. Since the excitability of a fiber is constantly fluctuating (Blair and Erlanger, 1933) the intensity of current that just suffices to block a given number of the spikes of a burst must be defined as the one that blocks that number in 50 per cent of a sufficient number of trials. Rarely will fewer than 12 trials (sweeps) suffice for a determination; usually many more are needed. Since the sweep cycles have occupied about 2 seconds and since in many of the experiments it has been necessary to make scores of determinations of blocking thresholds, the anode polarization often, indeed usually, had to be long continued, and therefore it became necessary to know how a block determined by anode polarization changes with time.

The results of an experiment planned with this in mind are collected in Fig. 2. One spike per sweep (1.82 sec.) was made to course along the fiber and the voltage needed to block these impulses was followed. The preparation had not previously been polarized but, when started, the polarization was maintained continuously although with each determination the voltage was adjusted to just blocking intensity. Every determination made during a 57 minute period is included in the graph. The polarizing voltage then was kept at the 57 minute level for a period of unnoted duration before starting an experiment to be described below. It is seen that the voltage needed to maintain a block diminishes with the time,* but that eventually the curve begins to run almost horizontally. One way of minimizing the changing effectiveness of the anode as a block, therefore, is to apply the polarizing current but postpone the experiment proper pending the attainment of this relatively steady state.

But, as will be seen, there is in the experiments as we have had to perform them another factor influencing the voltage needed to block, a factor

* It is stated (Schafer's *Text-book of physiology*, vol 2, p 498) that during the period of current flow the excitability at the anode, as tested by induction shocks, falls gradually to a minimum from which it then rises slightly. Recent determinations by Schmitz and Schaefer (1933) indicate that the change at the anode reaches equilibrium in about 0.5 sec. Our times are of a wholly different order of magnitude.

that is affected by time also, namely, the period of subnormal excitability following a tetanus. This, according to Gasser (1935), may last many minutes, whereas the longest interval permitted by our rotor was about 2 seconds. The briefer the tetanus the briefer and the less significant is the subnormality. To minimize this source of difficulty one might resort to very brief tetani and this has been done where possible. But it will be seen that even when the bursts consist of fewer than 15 rapidly repeated spikes the complication may not be eliminated; and 15 spikes do not suffice to carry facilitation to the maximum attainable. The best method would have been

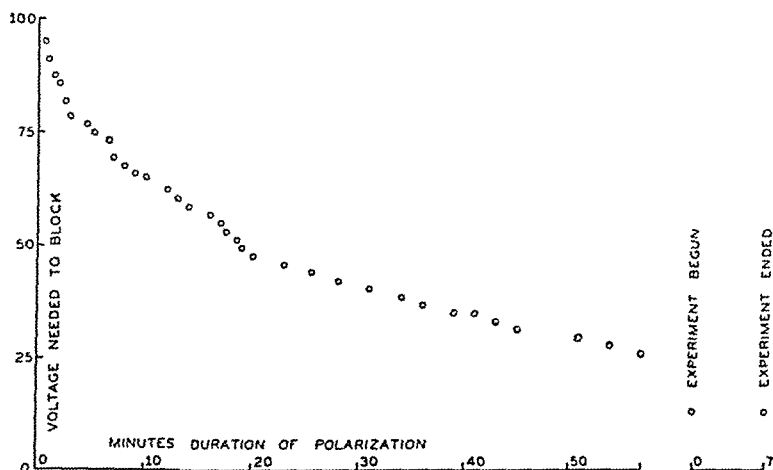


FIG. 2. Graph of the voltage needed to block one spike per sweep (i.e., one stimulus per 1.82 sec.), against the duration of the polarization. The initial current strength is assigned a value of 100. After an indeterminate interval (indicated by the break in the base line) during which the polarizing circuit remained closed, the experiment plotted in Fig. 3 was begun without interrupting the polarizing circuit; it occupied the time indicated here (0 to 7). During that experiment the current needed to block the one spike per sweep fell insignificantly.

to have used rest intervals exceeding the after-potential in duration through the employment of the hand-operated switches. To have done so, however, in experiments that required scores of determinations of the threshold polarizing voltage, would have increased enormously the risk of protracting them beyond the limits of constancy of the preparation. Indeed, as it was, not a few observations had to be terminated before completion, many of them owing to the ultimate onset of block at the polarizing cathode.

That there is some factor (undoubtedly the subnormal period) in addition to the anode polarization affecting determinations repeated every 2 sec. is indicated by the continuation of the experiment just described. Present observations were begun after the polarizing current had been flowing for considerably over an hour, and at a time when its effectiveness as measured by the voltage needed to block but the one spike per sweep had reached a more or less steady state (see Fig. 2). Tetani were employed now instead

of single shocks. Each tetanus consisted of 40 spikes at the rate of 120 per sec., the interval between sweeps remaining 1.82 sec. The quiescent intervals, therefore, had a duration of 1.49 sec. All of the first 59 of this series of sweeps were recorded excepting the first two and the data included in the graph, Fig. 3, are derived from the records. The progressive increase seen here in the number of spikes required for facilitation is very much more rapid than has usually been the case; possibly this is referable to the long period of anode polarization which had preceded (and was continuing through) this observation.* At first this increase in block proceeds fairly regularly with the time, but beyond about the 15th sweep the fluctuations

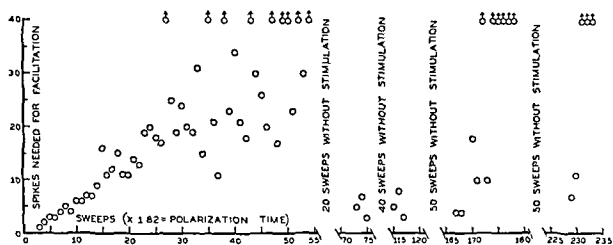


FIG. 3. Graph of the number of spikes required to effect conduction by facilitation, as affected by the time elapsing after establishing an anode block that just suffices at the outset to block the first spike only. The polarizing voltage remains constant throughout the experiment. With each sweep, excepting where otherwise indicated, the fiber is stimulated 40 times (at the rate of 120 per sec.). The circles attached to arrows signify that the 40 spikes of the sweep did not suffice to establish conduction through the block. After the last (the 233rd) sweep polarization was stopped and the block disappeared instantly.

in the number of blocked spikes from sweep to sweep becomes considerable. With the 27th tetanus the 40 spikes for the first time cease to suffice to facilitate to the conduction level, and then this situation recurs with increasing frequency as the experiment proceeds. The significance of this increasing fluctuation in the number of spikes blocked will be considered later.

At the moment we are concerned with the cause of the rapid and progressive increase in the number of spikes needed to overcome the block. Growing effectiveness of the anode of the polarizing circuit, at least as a potent factor, can be excluded on two counts. In the first place, the current needed to block one spike per sweep was the same at the end as at the start of this series of observations. Secondly, four times during the course of the experiment stimulation was stopped temporarily, for 20 sweeps, 40 sweeps, 50 and 50 sweeps, respectively, as indicated in Fig. 3, but the polarization

* In many experiments this "spontaneous" growth in the intensity of the block was so gradual that it was easily possible to determine satisfactorily the threshold blocking voltage at each level of block as it was changed experimentally.

was continued. Each period of rest immediately reduced the number of blocked spikes required to reestablish conduction—the performance became almost, though not quite, as good as it had been at the start of the experiment. But with the resumption of periodic tetanization, the anode polarization still continuing, the fiber very quickly lost again the ability to conduct. Now these fibers do not fatigue readily at the rates of stimulation employed here; therefore it becomes necessary to refer the rapid increase in the number of blocked spikes required for the reestablishment of con-

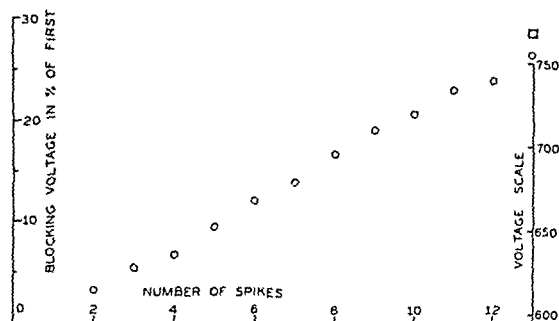


FIG. 4. Graph of the facilitation effected by blocked spikes, in relation to the number blocked. Stimulation was at the rate of 167 per sec., and therefore the intervals between circles are 0.6 msec. Ordinates (left), blocking voltage in per cent of the voltage required to block the first spike at the start of the experiment; (right), in scale divisions of the voltage divider. Abscissae, the number of spikes blocked. The ordinate for the square is the blocking voltage in per cent of the voltage required to block the first spike at the end of the experiment.

duction to some other cumulative factor, and this unquestionably is accumulating subnormality of excitability on the part of the postfiber.* But before discussing the influence of subnormality on these determinations an experiment on facilitation will be described in which it so happened that little or no correction was necessary for changes occurring with time.

Experiments on the effect of the number of spikes and of their rate, on the amount of facilitation obtainable. In Fig. 4 are plotted the results of an experiment in which the fiber was stimulated 13 times with shocks spaced at 6 msec., the

bursts being repeated about 30 times per min.† The voltage (readings on the voltage divider) sufficing for anode block of the first spike of a tetanus was 595 at the start of the experiment and 585 at the end. In other words there was relatively little change in the effectiveness of the polarizing current during the course of the experiment. Moreover, it was possible in this case so to adjust the polarizing voltage as to have conduction accomplished by any desired number of blocked spikes, regularly 1, 2 or 3, up to the limit of 13, and for considerable periods of time. The figure shows that the 13 spikes succeed in overcoming a block that exceeds by about 28 per cent the current needed to block the first spike; and though the curve is beginning to flatten, it is obvious that by no means had the maximum facilitation yet been attained.

* It will be convenient and appropriate to designate as prefiber and postfiber the parts of the fiber proximal and distal, respectively, to the block.

† This is the observation on temporal summation of multiple spikes that is referred to in a previous publication (Erlanger, 1939).

But in most of the experiments the apparent effectiveness of the polarizing current has constantly increased; and the preliminary experiment, it will be recalled, indicates that a part of this effect is referable to the overlapping of a tetanus and of the period of subnormality following in the wake of a preceding tetanus, an overlapping which could not be avoided, owing to limitations imposed by the machinery that had to be used in order to expedite determinations.

Let us now consider a specific experiment (see Fig. 5A and B) and indicate how these factors might be expected to affect the results obtained. To repeat, the threshold blocking voltage was determined for each successive change in the number of spikes blocked, and each of these determinations was immediately followed by a determination of the current strength needed to block the first spike of the tetanus. Other conditions here were (a) cycles recurring every 1.82 sec., (b) tetani consisting of 40 spikes, (c) at the three stimulation rates, which were 100, 50 and 200 per sec. in three successive series of tests, respectively, (d) tetani which lasted, therefore, 0.4, 0.8 and 0.2 sec. and (e) fell into whatever phase of the excitability cycle after-potentials that may have obtained 1.42, 1.02 and 1.62 sec. later, respectively. Under these conditions the tetani must all have begun during the subnormal phase of the postfiber; and though there is no exact information available on the basis of which one can estimate the degree of subnormality that actually obtained, one can assume from what is known, that in general the subnormality was greater the shorter the interval between these successive tetani. Therefore, with any given rate of impulses, the larger the number of impulses blocked the less would be the subnormality developing in the postfiber; and when all of the spikes are blocked there would be no subnormality in the postfiber.

In these experiments the effects of subnormality and of anode potential appear to sum. Thus when but one spike of a series is blocked, and subnormality in the postfiber is high due to the long tetanization periods, relatively weak anode polarization suffices to block. As more and more of the spikes are blocked by anode polarization, and the subnormality in the postfiber consequently becomes less, relatively more anode potential is needed to maintain the block.* Now when the voltage required to block, say, 20 spikes of these bursts of 40 repeated every 1.82 sec. is being compared with that required to block only the first spike of such a burst, the subnormality eventually developing in the postfiber under the first set of conditions would be less than that developing under the second. And it is obvious that the greater the degree of block, and possibly the slower the rate of stimulation, the number of the spikes remaining the same, the larger would be the error when the voltage that blocks the first spike is employed as a yardstick. This analysis, it will be seen, is borne out in general by the experimental data.

* Since subnormality and electropositivity are associated manifestations of a fiber, it is quite possible that this positivity does enhance the depressing effect of the anode of the polarizing current.

We turn now to a consideration of those data. They are plotted in Fig. 5, in two ways, namely, in A, with

Voltage to block N spikes—voltage to block the first spike,
and in B with

$$\frac{\text{Voltage to block } N \text{ spikes} - \text{Voltage to block the first spike}}{\text{Voltage to block the first spike}} \times 100$$

as ordinates, and with time as abscissae. Through the use of the second device it was hoped to achieve absolute results, but, in consonance with the foregoing considerations, it was found (as may be seen in Fig. 5, B) that determinations made late in a series are high, and that they are higher the larger the number of spikes blocked. In order to make this obvious in the graphs the individual determinations have been numbered in the order in which they were made. The first method mentioned above yields only relative results since with it the base line is constantly shifting, but they nevertheless are instructive. The curves show (1) that as many as 30 successive spikes can sum their effects across a block and (2) that the facilitation accomplished by the same number of blocked spikes at the rate of 200 per sec. may be six to eight times as great as that accomplished by spikes at rates of 50 and 100 per sec.

In order to get better information with regard to the effect of rate of stimulation on facilitation, experiments of a slightly different type were performed. Instead of making long series of determinations at each rate of stimulation, as was done above, the current strength was determined that was necessary to block a given, and the same, number of spikes at each of a few rates of stimulation, and the order of the determinations was staggered so as to make it possible to recognize any cumulative effect in time. In the case illustrated in Fig. 6, each tetanus consisted of but 15 spikes, and a

FIG 5 A Graphs of the relation of the voltage needed to block N spikes minus the voltage needed to block the first spike (ordinates), to the number of spikes blocked. The tetanus consists of 40 spikes. Circles—5 msec intervals between spikes, dots—10 msec intervals, triangle and squares—20 msec intervals. There was an interruption between the two sets of determinations at the 20 msec intervals.

B is the same as A except that the ordinates are

$$\frac{\text{Voltage to block } N \text{ spikes} - \text{Voltage to block the first spike}}{\text{Voltage to block the first spike}} \times 100$$

In the case of the 10 msec curve the voltage, through observation 27, was set arbitrarily and the number of blocked spikes determined, the spread of a reading between two spikes therefore is of no significance. Thereafter, and for all other curves, each reading is the threshold blocking voltage for the indicated number of spikes, and the width of the spread then is indicative of the diminishing facilitation per spike. Beyond about 25 spikes this spread is apt to extend out to the last, the 40th, spike, as indicated by the arrows. In many cases, however, no note was made of this spread.

current strength that blocked 10 to 11 of these was compared with the current strength that blocked 1 of them. The rates of stimulation used were 200, 167, 100 and 50 per sec., and the cycle period was 2.15 sec. The determinations at the different rates were made as expeditiously as possible. Otherwise the experimental procedure was as above.

During the course of the experiment the current strength that blocked one spike fell from an average of 621 in the first series of determinations (see the numbered points in the graph) to 286.5 in the 8th series. In Fig. 6 the results are plotted in the two ways mentioned above. When plotted by

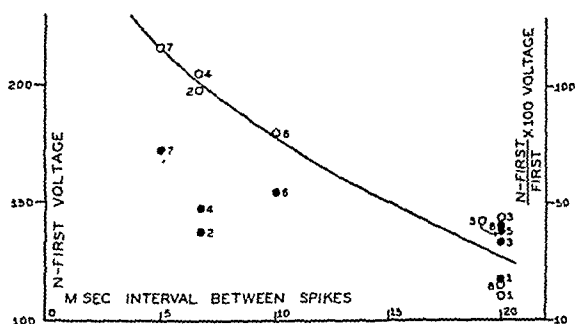


FIG. 6. Graph of the relation of the voltage needed to block N spikes minus the voltage needed to block one spike (circles and curve), and of

$$\frac{\text{Voltage to block } N \text{ spikes} - \text{Voltage to block the first spike}}{\text{Voltage to block the first spike}} \times 100$$

(dots), to the number of spikes impinging on the block per unit of time. The numbers indicate the succession of the determinations.

the N -First method the points fall nicely about a smooth curve with little if any evidence of an effect of the sequence of the determinations. It is seen that the facilitation effected by the 10 to 11 spikes at the rate of 200 per sec. is greater by about 71 per cent than that effected by the same number of spikes at the rate of 50 per sec. The shape of the curve is such as to indicate that facilitation would still be in evidence at very much slower rates than 50 per sec. and that it would increase rapidly at rates higher than 200. Qualitatively, facilitation by multiple blocked spikes is demonstrable with intervals of 100 msec.

The graph based on the per cent of the ratio, $\frac{N\text{-First}}{\text{First}}$, gives, again, very

marked evidence of the disturbing effect of elapsed time, but when this is taken into account this method of plotting also shows that the facilitation is greater at higher than at lower rates of stimulation.

A fact of some interest in connection with these observations on the temporal summation by many successive spikes is that never are there any

interposed blocks in a tetanus, once facilitation has succeeded in overcoming the initial block. In view of the fact that the excitability of fibers is constantly fluctuating spontaneously through a range that usually amounts to about 5 per cent of the threshold, this unfailing response of the postfiber to the successive spikes, once it has begun to respond, signifies that with the first response something has transpired that immediately lowers the threshold of the postfiber by an amount that is greater than the spontaneous fluctuation in threshold. Undoubtedly what happens is that the successive spikes of the prefiber fall into the supernormal phases left in the postfiber by preceding responses.

Another factor which possibly assists in maintaining transmission, once the block has been overcome, is the background of a tetanus. Gasser's illustrations (1935) show that the negative after-potential in tetani comparable in rate and number of stimuli with those employed by us, may grow as the tetanus proceeds, so that both the peak potential and the potential of the background may rise, though the spike height remains constant. Though all spike potentials on this basis would be expected to send into the postfiber the same number of current lines, the growing negative after-potential, by continuously and increasingly polarizing the postfiber cathodally, would be expected to lower its threshold continuously, and so increase the effectiveness of the blocked spikes.

In the average experiment it is possible to adjust the blocking voltage so that a given number of spikes will be blocked regularly through a shorter or longer series of successive sweeps, as long as the number of spikes blocked is not in excess of about 10 to 15. Beyond that number the successive sweep pictures at any setting of the polarizing current exhibit varying numbers of blocked spikes, the variations being small at first but eventually very wide, so that a setting of the current that blocks, say, 20 spikes in one sweep, may block 40, or whatever the number available may be, in the next. These fluctuations are an expression of the spontaneous variation in the excitability of a fiber. As long as the summation increment per spike is in excess of this spontaneous fluctuation the successive pictures will be stable; and the instability will appear and increase as the successive summation increments fall to and below the range of the spontaneous fluctuations in excitability.

Difficilitation. When, with the rotor, a fiber is made to carry two relatively short bursts of spikes while it is being so polarized anodally at the lead that the spikes of the first burst are conducted through by facilitation, a spacing of the two bursts from each other can be found such that the second will require more spikes to overcome the block than the first (difficilitation), whereas a shorter spacing of the bursts can be found at which none of the spikes of the second burst will be blocked.

When, under conditions otherwise the same the first burst (through the hand-operated switch) is made a long one (2 or more sec.), a short intercalated period of no impulses (intervals of 0.5 to 1.0 sec. have been used) may be followed by block. If it is, recovery *ordinarily* will not occur as long

as the second tetanus is continued (the longest carefully checked period of second tetanization has been 43 sec.). But if this second tetanus is interrupted for a period of 6 or more sec. after it has been running for some sec. (5 or more), conduction through the block *usually* begins either at once with the resumption of tetanization, or after a relatively short period of facilitation. Apparently the second (but blocked) tetanus tends to maintain the difficilitation "indefinitely," whereas interrupting it temporarily permits of rapid recovery usually without the need of facilitation.

It may be inferred from the above summary that there must be many conditions affecting quantitatively the difficilitation obtained, such as the number of spikes needed for the facilitation of the first burst, the number of spikes constituting the first burst, the rate of stimulation, the intervals between the bursts, the changing effectiveness of the continuous polarization, fatigue, etc. The effects of these qualifying conditions have not been systematically investigated. Those conditions that can be controlled have been altered only in so far as has seemed to be necessary in the effort to determine which are fundamental to the development and continuance of difficilitation.

A description of the essential parts of an experiment in which both the rotor-operated and the hand-operated switches were used will serve to illustrate both the techniques employed and many of the characteristic reactions. There are many confirmatory observations, but none in which it has been possible to gather information from one and the same fiber under comparable conditions through the use of both the rotor- and the manually-operated switches, giving a reasonably wide range of stimulation and of rest periods. The stimuli were delivered at the rate of 74 per sec. With the rotor, the cycles recurred every 2 sec. The first burst (see Table 1) consisted of 23 or 24 spikes, and therefore lasted about 0.32 sec.; the second burst consisted of 15 spikes. At the outset the blocking potential was adjusted so that about two spikes were required for conduction in the first burst.

Table 1. *Difficilitation by spikes. A second tetanus requires more spikes to overcome the block than a first.*

Record no.		1	2	3	4	5	6	7	8	9	11
No. of facilitating spikes in	1st tetanus	3	2	2	1-2	5	5-6	2-3	6-7	5	6-7
	2nd tetanus	7	10	9	10	10	9	9	10	10	10

By trial the two bursts were spaced by an interval that gave the maximum difficilitation obtainable under the circumstances; this interval was 0.52 sec. Narrowing the interval not only stopped difficilitation, it actually eliminated the need of facilitation in the second burst, the postfiber then responding to the first impulse of the second burst in the prefiber, but this limiting separation was not recorded. As may be seen in the table, the

number of spikes needed to establish conduction during the conditioning burst increased irregularly from about 2 to 6 or 7; for the testing burst it varied between 7 and 10. There was no obvious relation between the number of spikes blocked in the two bursts of a cycle, but with this separation the second burst always required more spikes to reestablish conduction than the first.

These particular conditions, therefore, yielded definite difficilitation. It can be shown, however, that the conditions here were far from the optimal for the development of the phenomenon. The important limiting circumstance was the duration of a cycle,—2.0 sec.; for at the optimal separation of 0.52 sec. between the first and the second bursts, the interval between the second and the first bursts was 0.6 sec. In other words, the latter interval was but little longer than the former, a state of affairs which must have resulted in making the difficilitatory effect of the second burst on the first almost as great as that of the first on the second. Moreover, this situation must have created a tendency toward the accumulation of difficilitation (subnormality), such as was noted in the first section of this paper. The more or less progressive increase in the number of the spikes needed for the facilitation of the first burst, mentioned above, probably was due likewise to this circumstance.

Then the hand-operated switches were employed. Here a "cycle" involved many sweep times, and it became necessary to develop a special technique in order to keep track of elapsed time and to observe transient phenomena. The spot occupied about 1.6 sec. in its transit across the face of the tube, and the returning intervals were about 0.4 sec. During each of these intervals a new frame of film was brought into position so that the whole of every sweep could be photographed. Though the pictures were thus taken discontinuously, the experiment continued without interruption. Each procedure was so timed that changes in imposed conditions occurred during a sweep, so that the immediate consequences could be observed.

The essential parts of one series of observations are reproduced in Fig. 7. The rate of stimulation and the amount of facilitation in the conditioning burst were about the same as in the first part of the experiment, the polarizing current being such that initially 7 spikes were required to overcome the block (sweep 1). This initial tetanus was continued without interruption for 6.3 sec. Then (sweep 4) stimulation was interrupted 0.68 sec. With the resumption of stimulation it is seen (sweep 4) that the impulses are blocked at the polarized locus: the change in the picture of a spike seen here is typical of block at the lead, in that the spike diminishes in height and becomes monophasic. This block persists through 5 sec. of stimulation. Stimulation then was stopped again (sweep 7) and was not resumed until 6 sec. had elapsed. It is seen (sweep 10) that with the resumption of stimulation the block has disappeared; not only this—conduction through the block now is accomplished without any facilitation. And though stimulation now was continued for 18 sec. there was no return of block. Since the polariza-

tion was maintained at a constant level throughout this experiment, this last step shows that the previous block could not have been due to increasing effectiveness of the anode polarization. It shows also that fatigue was not the determining factor in the maintenance of this block.

In the same experiment under similar conditions a tetanus lasting 8 sec. and a 0.72 sec. period of no stimulation were succeeded by "permanent" diffcilitation also (it was followed for 11 sec.). Then an interpolated period of no stimulation lasting 14 sec. removed this block as above, and conduction continued thereafter until the experiment was discontinued, 16 sec. later.

Similar responses have been obtained in a number of preparations, but

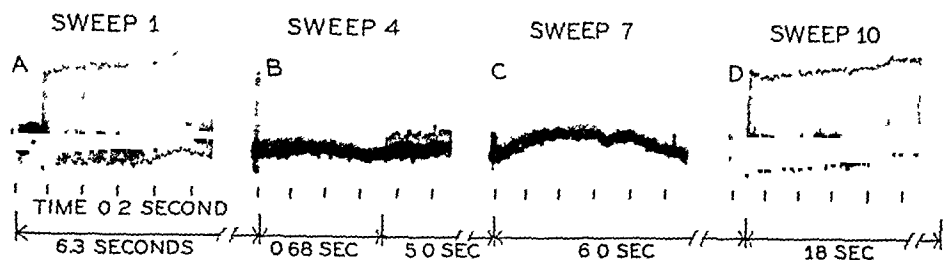


FIG. 7. Facilitation and diffcilitation. Sweep 1 starts with the spike blocked at the lead. The 8th spike conducts through by facilitation; the spike not only becomes higher, but it becomes diphasic also. The positive, down, deflection during block is the shock artifact; at other times it is this plus the positive phase of the spike.

Sweep 4 begins with full spikes which have been continuing without interruption through sweeps 2 and 3 (not reproduced). During this sweep stimulation is interrupted for 0.68 sec. With its resumption the impulses are blocked.

Sweep 7, as it starts, shows this block still continuing. Then stimulation is stopped, and not begun again until—

Sweep 10. Here it is seen that with the resumption of stimulation the fiber conducts through again and without the facilitation needed at the start of sweep 1.

To gain space the sweep pictures have been cut on their left ends. The variations in spacing of the spikes are due to sweep irregularities and are without significance.

they cannot be demonstrated in all. Moreover, even in the case of the same preparation such results as are described above may be transient and inconstant. Extracts from the protocols of another experiment may be cited in order to illustrate an occasional variation from the rule.

In this case the switch was operated by hand. The rate of stimulation was 62.5 per sec. Only one fiber was responding. (i) Anode polarization was adjusted so that from 3 to 8 spikes were required for conduction. (ii) Without altering the polarization the preparation was allowed to rest for a time and then (iii) It was stimulated tetanically for a period of 8 sec. All impulses were conducted through. (iv) Then stimulation was stopped for 1 sec., and, (v) When stimulation was resumed all impulses were blocked. This period of stimulation, with continuous block, lasted 11 sec. (vi) Then stimulation

was stopped for 10 sec. (vii) On resuming stimulation block still was in evidence and persisted for 3 sec., when the impulses began to be conducted through.

It should be added that at another time in this experiment it had been possible repeatedly to demonstrate the usual result, namely, the immediate termination of "difficilitation" by a rest period. It may be worth mentioning also that in order to obtain facilitation and difficilitation in the present case it was necessary to add calcium to the Ringer's solution; this had not been necessary in the case of the first experiment cited above.

In these experiments conditions do not remain constant long enough to permit of the collection of satisfactory controls. This has made it impossible thus far to determine specific limits for the significant phases of the processes under investigation. Some of the extremes that have been observed have been given above, but it should be distinctly understood that they do not represent limits. An important qualifying factor must be the grade of the initial block and we have tried to hold this at the convenient level where 2 to 8 spikes were required for conduction. Under these circumstances we have seen "indefinite" difficilitation in response to a silence of 0.5 sec. following a tetanus of 2 sec., and in the same experiment no difficilitation following the same silent period after a tetanus lasting 1 sec.

Information regarding factors determining the duration of difficilitation is particularly incomplete. In the first place, we do not know how long difficilitation would have lasted (beyond 43 sec.) had the second tetanus been allowed to continue indefinitely. A 5 sec. interruption of tetanization of the prefiber usually has sufficed to allow impulses to pass when stimulation was resumed. But we have seen the difficilitation continue after a 24 sec. interruption of stimulation, and this in an experiment in which in several trials the fiber had conducted through immediately after very much shorter intercalated periods of rest.

Observations such as these should put us on our guard against a possible fallacy in the interpretation of the data. A period of no spikes may not curtail difficilitation. Instead, it is possible that a subnormal period following the first (the conditioning) burst has needed for its complete subsidence a period longer than the second tetanus, but shorter than the second tetanus plus the subsequent silent period. If the latter were the case the disappearance of the subnormal period could be disclosed only by supplying testing spikes during the second "silent" period. In other words, this question can be settled only by collecting a large number of comparative observations on the time required for the disappearance of the block initiated by momentary intermission of a tetanus under two conditions, namely: (i) when the second tetanus is continued indefinitely and (ii) when only occasional testing spikes are made to impinge on the blocked locus. Since we do not have that information all that can be said now is that in the present set of observations we have never seen a posttetanic block disappear while the spikes of a *second* tetanus were playing on it.

How may facilitation and diffcilitation be accounted for? Facilitation undoubtedly is referable to an electrical effect exerted on the postfiber by the axon spike of the prefiber acting across the local discontinuity in the fiber. The known facts all support this view. Thus a subthreshold electrical shock immediately lowers the threshold, which then returns to normal through a period of over 50 msec. (Blair, 1938); and a series of subthreshold shocks as widely spaced as 20 msec. may sum their effects to cause fibers eventually to discharge (Gasser, 1938). Exactly the same effects with similar time relations are exerted on a postfiber by a blocked spike (Blair and Erlanger, 1939), and, as described in the present paper, by a series of blocked spikes.

Diffcilitation (or at least the first of its phases, assuming that there are two) finds a simple explanation in the subnormality of the postfiber into which the latter passes subsequent to the termination of a conditioning tetanus; the block that has been overcome by facilitation is easily reestablished, and is reestablished by failure of the spike of the prefiber, acting electrically, to attain the threshold of the subnormal postfiber.

The question now arises, is there a second phase to diffcilitation which accounts for the apparent fact (discussed above) that once diffcilitation has been induced the postfiber will not ordinarily carry impulses during the continuance of the tetanus in the prefiber? To what might such a protraction be due? Subnormality of the height of the spikes in the prefiber can be eliminated, since the height of the blocked spikes of the second tetanus is the same, as nearly as it can be measured, as that of the spikes blocked by anode polarization at the start of the conditioning tetanus before conduction through the block has been established by facilitation.

Is it, then, referable to some depressing influence exerted by the tetanized prefiber on the postfiber? Two possibilities suggest themselves in this connection. (i) The prefiber not only supplies spikes, but these spikes in addition ride on the background of the negative after-potential. The negativity of the after-potential, like that of the spike, must lower the threshold of the postfiber through electrical action. If, therefore, the background of a second tetanus following a first after a few seconds were lower than that of the first it is quite conceivable that this might suffice to maintain a block which otherwise would have disappeared. However, facilitation, as has been seen, can lower thresholds quite materially, and it seems rather unlikely that any deficit there might be in the negative after-potential background of such a second tetanus would be sufficient to overcome the facilitating effect of the spikes. It does not, however, seem profitable to pursue this idea further, since data on the background potential of a tetanus initiated during the positive after-potential following a previous tetanus seem to be entirely lacking.

(ii) The other possibility that occurs to us is that repeated subthreshold spikes (and electrical stimuli) may maintain continuously the elevated threshold that obtains in the postfiber during the subnormal phase of its excitability cycle. There is available no specific information bearing on

this suggestion, either, and it may, therefore, be somewhat gratuitous to say that such a depressing action would constitute inhibition, and would put inhibition on an electrical basis

There is to be considered also the possibility that rest hastens recovery from difficiltation by permitting the prefiber to deliver impulses which are stronger than those developed during a continued tetanus. This thought is suggested by the fact that the initial spike of the third tetanus in sweep 10 of Fig. 7 is higher than the first full spikes of sweep 1 (though the height of the spikes of sweep 10 quickly decrement before starting a treppe). But the prefiber contributes relatively little to the recorded height of a spike that is conducted through, so that the increase seen here while the spike is blocked, must be a contribution of the postfiber, primarily. If, however, the first spike of the prefiber is increased to the same extent as is the spike of the postfiber it still might be possible to refer the recovery from prolonged difficiltation that occurs after a period of no stimulation to that rather than to anything that is transpiring in the postfiber.

Extinction. Dusser de Barenne and McCulloch recently (1935 and 1939) have described the phenomenon of "extinction" which they define as (p. 320) "a diminution or absence of response on repetition of stimulation of a 'motor' focus within an interval longer than that required for facilitation." These authors believe that extinction is not due "merely to the passage of a stimulating current" since it does not occur unless the electrical stimulation elicits a neural response. Extinction then is a process that takes place at a synapse and has the same electrical basis as the "difficiltation"* we have demonstrated at a discontinuity in a nerve fiber. It is of interest to add that extinction is associated with local electropositivity.

Recruitment and disbandment. That the blocked spikes act electrically to produce facilitation and to influence difficiltation is indicated by experiments we have made on the responses of fibers to direct electrical stimulation. Here the stimuli are shocks and the "discontinuity" consists of the junction of the electrical circuit with the nerve fiber, whereas in the former case the stimuli are spikes acting across a discontinuity in the fiber. Comparative observations have been made only with the rotor operated keys. Pictures obtained by the two methods, showing recruitment (or facilitation) and "disbandment" (or difficiltation), are identical in every respect save one: when the relevant events transpire at the stimulating electrodes no part of the spike is visible during the periods when the shocks are below the threshold, only the shock escapes appearing in the records, whereas when there are impulses which are blocked at the lead, the spike potential is recorded, but in reduced amplitude, as well as the shock escape.

* We prefer the term "difficiltation" to "extinction." For though facilitation and extinction (difficiltation) are not, as these authors state, antagonistic phenomena (since they are successive), they are so as regards the effects they produce. Thus a block in a fiber that has been overcome by facilitation is reestablished by difficiltation. And in the case of the central nervous system the one process increases action, the other decreases it.

Though the results obtained in the present experiments might have been anticipated on the basis of what is known regarding the excitability cycle, they do throw light on some questions of interest. One set of observations, therefore, will be described and illustrated (see Fig. 8 and Table 2). The polarizing current here is dispensed with. The preparation contained three fibers that conducted to the lead. The spike of fiber A was high and recorded

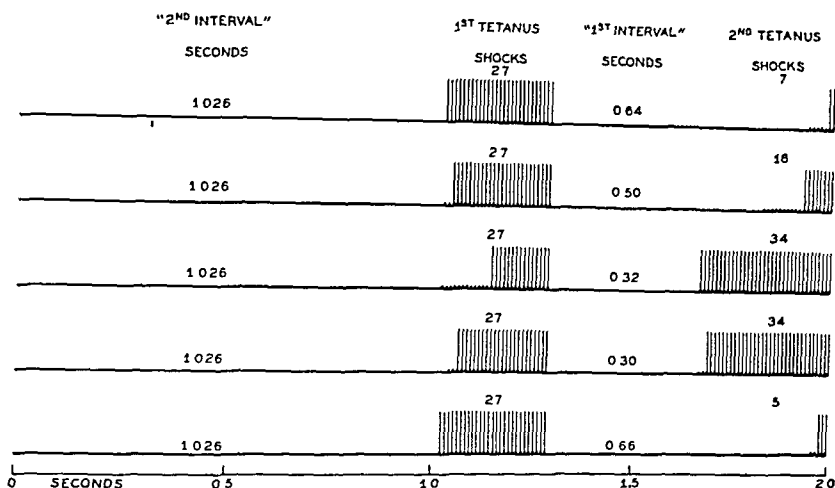


FIG. 8. Diagram showing recruitment (facilitation) and "disbandment" (difficilitation) in fiber A (table 2), effected through direct electrical stimulation. Each horizontal line is a conventionalized, but accurate reproduction of a cycle. For the sake of clearness the second interval between tetani is placed ahead of the "first" tetanus. The rate of stimulation is 80 shocks per second. Each shock is indicated by a dot; those surmounted by lines reached the threshold of the fiber.

monophasically, that of B diphasically, while fiber C responded somewhat irregularly with a low monophasic spike. C was difficult to recognize in certain situations; consequently its behavior will not be considered. It may be added, though, that when it responded it was always by facilitation, never with difficilitation. The rotor-operated switches were employed to deliver two tetanic bursts of shocks at the rate of 80 per sec. The cycle occupied 2.15 sec. The first burst consisted of 27 shocks; the number in the second varied as the interval between the first and second bursts changed, so that the intervals between the second and the first bursts in the successive cycles remained essentially the same throughout (1.026 ± 0.04 sec.). The shock employed at the start was just about threshold for fiber A so that in the first burst of a cycle A usually responded to the first shock.

The diagram (Fig. 8) shows clearly that in the case of fiber A difficilitation (negative recruitment or "disbandment") is greatest when the interval between the first and the second bursts is 0.5 sec., and is nearly or quite absent both at narrower (0.3 sec.) and at wider (0.66) intervals. In Table 2 it may be seen that fiber B behaves similarly, though not so obviously. The

resting threshold of B was higher than that of A, as is indicated by the larger number of shocks needed to reach the threshold during the first burst. Indeed, in most of the trials so many were needed that the number of effective shocks in the testing burst was fewer than the total in the conditioning burst, so that difficilitation, if present, had no opportunity to

Table 2. Recruitment (facilitation) and "disbandment" (difficilitation) by electrical stimulation in two fibers, A and B.

Record No.	Time bet. 1st & 2nd Tetani	Time bet. 2nd & 1st Tetani	Shocks in 1st Tetanus	Shocks in 2nd Tetanus	Facilitating Shocks in 1st and 2nd Tetani			
					Fiber A		Fiber B	
					1st	2nd	1st	2nd
1	sec. 0 64	sec. 1 073	27	7	0	1	13	7+
2					0	7+	14	7+
3					0	4	14	7+
4					0	3	9	7+
5					0	7+	14	7+
6					0	7+	14	7+
7	0 5	0 983	27	18	0	9	23	2
8					1-2	11?	27+	11
9					1	9	11	13
10					0	12	14	14
11	0 32	1 060	27	34	14	0?	25	0
12					13	0	27+	34+
13					13	0	27+	25
14					16	0	23	0
15	0 3		27	34	1-2	0	27+	25
16					1-2	1-3?	17	0
17					1-2	0	27+	25
18					1-2	0?	13	0
19	0 66	0 989	27	5	0	2	4	5+
21					0	0	10	5+
22					0	2	3-4	5+
23					0	0	6	5+
24					0	3	13	5+
25					0	0	9	5+
26					0	1	13	5+
28					0	0	10	5+

"Disbandment" is indicated where the second tetanus requires more shocks for effective stimulation than the first. A plus sign signifies that the total number of shocks available did not suffice to reach threshold. When, in such instances, the number of shocks in the second tetanus is smaller than the number in the first it is not possible to know whether difficilitation would have obtained. The decrease in the number of shocks needed for recruitment in observations 15 to 18, as compared with observations 11 to 14 (first tetanus of fiber A), was determined by a slight increase in shock strength; it was made in order to reduce the increased number of shocks required for facilitation, an increase due probably to enhancement of subnormality by the increase in the number of spikes in the second tetanus.

exhibit itself. The few instances where the number does suffice for stimulation, indicate that the maximum of difficilitation occurs at about the same interval between bursts, namely, 0.5 to 0.66 sec., as in the case of fiber A.*

It is obvious then, that both subthreshold shocks and axon spikes that become effective through facilitation during a first tetanus act alike in respect to facilitation and to difficilitation. Now in the case of direct electrical

* This description omits consideration of certain unessential, but interesting features.

stimulation a background potential in a prefiber (here represented by the coil circuit) does not obtain. It follows that where impulses are impinging on an anode block, a *reduced background potential* in the tetanized prefiber is not an essential condition for the development of *difficilitation*. And it may be concluded that *difficilitation*, in its initial stages at least, is determined only by subnormality of the postfiber.

SUMMARY

At a discontinuity (block) in a nerve fiber created by anode polarization, a discontinuity that can be graded by varying the strength of the polarizing current, it is possible to demonstrate and to gauge the facilitation effected by blocked action potentials and to demonstrate a phenomenon, designated *difficilitation*, which probably is identical with "extinction."

The facilitating action of spikes at such an artificial synapse increases with their number and with their frequency. The facilitation per impulse declines slowly with the number, but is demonstrable through at least 20 impulses. The facilitating action has not attained its maximum at impulse rates as high as 200 per sec., nor its minimum at rates of 10 per sec.

Once, in a tetanus, a spike succeeds in passing a block, all of the succeeding spikes of that burst also will pass, notwithstanding the spontaneous variations in the excitability of a fiber. This effect is attributed to the supernormality that follows immediately the response of the postfiber.

Difficilitation is demonstrable, when conduction has been established by facilitation, by interrupting this conditioning tetanus momentarily, the optimum period of interruption (under a specific set of conditions) being in the vicinity of 0.5–0.6 sec., when, upon resumption of the tetanus, it may happen that spikes will no longer pass from the prefiber to the postfiber (i.e., across the local discontinuity) as long as the tetanus continues. (Observations on this interruption of conduction have not been continued longer than 43 sec.) But interrupting the second tetanus for a period of 6 sec. seems to permit the block to disappear.

Difficilitation, at least in its initial stages, is regarded as a manifestation of the subnormal phase of the excitability cycle in the postfiber a cycle that is started by the momentary interruption of tetanic stimulation; the spikes of the prefiber, with the resumption of tetanic stimulation, then no longer reach the threshold of the postfiber.

Exactly the same phenomena of facilitation (recruitment) and of *difficilitation* (negative recruitment or "disbandment") are demonstrable at the site of stimulation of a fiber with induction shocks, and it is concluded that both facilitation and *difficilitation* result from the spike's action as an electrical stimulus to the postfiber. This holds not only with regard to the artificial synapse in the nerve fiber but, by inference, with regard to the actual synapse, as well.

The protraction of *difficilitation* which *seems* to be determined by con-

tinued tetanic stimulation of the prefiber is discussed, but no conclusion is reached regarding processes that might be determining it.*

* Since this paper was submitted for publication there has appeared a paper by Lorente de N6 (*J Neurophysiol*, 1939, 2 402-463) dealing with some of the questions we have had to consider, such, for example, as the extension of after-potentials beyond blocks. Unfortunately it will be impossible now to take further cognizance of Lorente's paper.

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The protraction of difficilitation which *seems* to be determined by con-

upon termination of the stimulus, when the ordinary saying of the alphabet was immediately resumed. The repeated letters were slightly distorted, and their rate of production was perhaps slightly slower than the rate at which the patient spoke the rest of the alphabet. The phenomenon is exemplified in the following quotation from the patient. The italics represent the application of the stimulus "A B C D E F G H H H H H H I J K L M N N N N N N N O P Q R R R R R R S T U V W X Y Z." The performance could be repeated at will, and was carried out eight or ten times.

The patient, on questioning, said that she was fully aware of what was occurring, but was unable to check herself. No feeling of discomfort or other special sensation was experienced. The patient's condition was excellent throughout. Because of the rather excessive amount of electrical stimulation which had been given, it was considered best to ter-

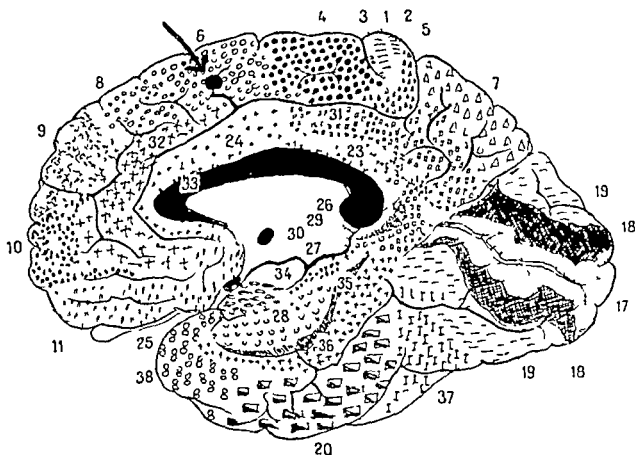


FIG 1 The approximate position of area X is indicated by the black disc (arrow)

minate the procedure without undertaking the numerous additional tests which might have been made.

After cauterization, area X was excised to a depth of about 0.2 cm, the entire specimen measuring about 1 cm in diameter. After the excision, stimulation of the exposed white matter, and of the cortical edges, failed to elicit the phenomenon. The specimen was fixed in formalin. Sections stained with hematoxylin and eosin and examined by Dr. Abner Wolf showed no changes which could not be explained by the surgical procedures.

Although the observation has, thus far, been made in only one case, it is none the less real. A point which must not be lost sight of is that the brain was not strictly normal. Not only was there a convulsive state, but also the bone of the vault was extremely thick, and there were two veins, one or both of which were abnormal. In addition, one of these veins had been ligated prior to the stimulation. The ligation, as such, produced no noticeable symptoms, at least nothing immediate.

These considerations bring up the question of whether such an area as X can act upon normal brain tissue, whether an abnormality must be present in a zone for X to influence it in this way, or whether X itself must be abnormal for the effect to occur. Such questions as these may find answers in further study.

DISCUSSION

No known part of the speech zone lies near area X. The nearest cortical part, Broca's area, is 8.5 cm. away, as measured directly through the substance of a slightly atrophied brain fixed in formalin. Therefore, apparently normally acting cells of the brain were influenced, and caused to act abnormally, by stimulation of other cells a considerable distance away. The particular form of induced abnormal activity was one in which certain cells were thrown into function again and again. It was as though the nerve impulse were imprisoned in a given cell group, able to activate that group only, and unable to pass to another group. The situation recalls the "closed circuit" theory, propounded by Kubie (2) and others (3, 4, 5, p. 152) and elaborated by Lorente de Nó. (7), and can be analyzed in terms of circuit reactivation.

SUMMARY

During the electrical exploration of a human cortex, an area was found which when stimulated, produced perseveration of speech. The area (area X) lay on the mesial side of the left hemisphere, in area 6, probably just above the junction of that area with the posterior part of area 32.

The patient, under local anesthesia, said the alphabet. At each application of the stimulus, and throughout the period of stimulation, the letter the patient was saying was repeated over and over again. The perseveration ceased instantly when the stimulus was stopped.

Area X is far from any known part of the speech zone. It influenced the function of distant neurons, and in such a way that these neurons were thrown into action again and again, as though the impulse were imprisoned in a given cell group, able to activate that group only, but unable to pass to another.

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The drug solutions were sometimes applied to the cortex in small squares of "spot test" paper, each square was 4 mm on the side. At other times application was made by dabbing or painting with a fine camel hair brush. The following drugs were used: eserine sulphate (Burroughs Wellcome & Co.), acetylcholine bromide (Eastman Kodak Co.),

atropine sulphate (Merck); pilocarpine hydrochloride (Merck); the drugs were dissolved in Ringer-Locke's solution, containing 0.02 per cent NaHCO_3 .

Determinations of the pH of solutions. Determinations of the pH of the Ringer-Locke solution and of the drug solutions were made with a Beckman pH meter (glass electrode). The pH values are as follows:—

Ringer-Locke.....	7.3
1 per cent eserine in Ringer-Locke.....	6.6
1 per cent ACh in Ringer-Locke.....	7.3

The close similarity of the pH of 1 per cent ACh in Ringer-Locke's solution to that of Ringer-Locke's solution itself may be explained by the buffer action of the latter solution on the slightly ionized HBr of the acetylcholine bromide. The Ringer-Locke's solution was brought with 0.1 *N* HCl to 6.6, the pH of 1 per cent eserine, and was used as a control for eserine.

Controls with saline. Any possible electrical effects through conductivity changes between the electrodes were controlled by preliminary irrigation with saline and by the application of a paper square soaked in saline. These procedures yielded none of the effects observed by the drug applications.

RESULTS

For purposes of analysis and description of the effects of the drugs on the electrocorticogram we have defined the three following classes of waves, setting in each class arbitrary limits on period: (i) *Slow waves*; period 0.1–0.75 sec. (s, Fig. 1A). (ii) *Fast waves*; period 0.025–0.09 sec. (f, Fig. 1A).

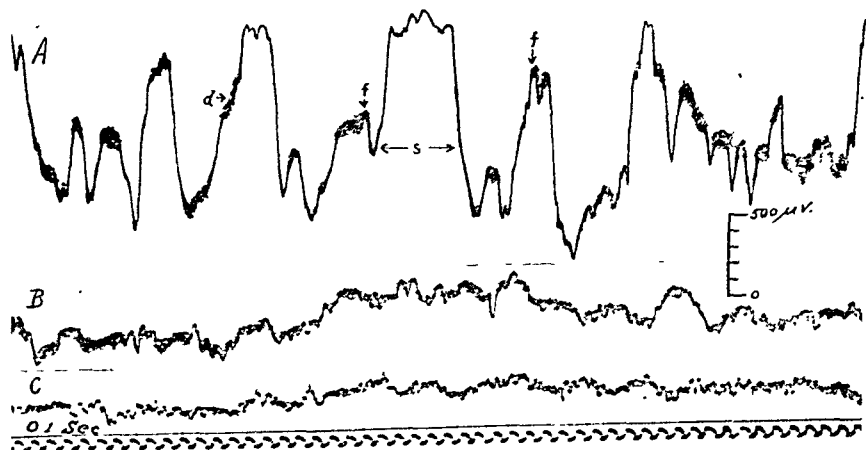


FIG. 1. Effects of eserine on electrocorticogram. Rabbit. Dial. Electrodes 10 mm. apart, one anterior, other posterior on cerebral cortex.

A. Before eserine; s, slow wave; f, fast wave; d, dot wave.
 B. 1 min. after application with small brush of 1 per cent eserine to cortex between electrodes. Reduction in amplitude of slow waves.
 C. 4 min. after 1 per cent eserine. Progress of eserine effect.

(iii) *Dot waves*; slight discontinuities in sweep of fluorescent spot; period 0.01–0.02 sec. (d, Fig. 1A).

Effects of eserine. The application of 1 per cent eserine to the cortex causes, within 1–2 min., a progressive reduction in amplitude of the slow waves and of the large fast waves; at the same time the small fast and dot waves become more apparent, possibly because of the reduced amplitude of the slow waves (Fig. 1 and 2). The above effects continue for approximately 30 min. after application of eserine. Accompanying the above electrical changes it is usually possible to observe motor phenomena, indicating cortical stimulation: these include muscular tremors and twitchings; they are more marked from eserine on the motor cortex of the cat, when the opposite fore- and hindleg are rigidly flexed and exhibit tremors (cf. Miller, 1937).

Control observations on the possible influence of pH differences were made by applying, to the cortex of the rabbit and cat, Ringer-Locke of the same pH as 1 per cent eserine, namely 6.6; this was found not to yield the characteristic effects of 1 per cent eserine, there being no detectable change in the potentials. Dusser de Barenne, McCulloch and Nims (1937) have shown that lowering the pH of the cortex itself reduces the electrical activity. The absence of change in potentials from reduced pH in our experiments may be attributed to the circumstance that the solutions were applied to the cortex in minimal quantities; these would mix with the larger volume of the cortical fluid, which would afford considerable buffering. Thus, in our experiments, it appears unlikely that the pH of the cortex itself would be changed. Hence the effects of eserine application may be regarded as due to specific drug action and not to lowered pH.

Effects of ACh on the non-eserinized cortex. As contrasted with the powerful effects of ACh on the eserinated cortex to be described presently those on the non-eserinized cortex are slight. In a few experiments, in which the cortex of the rabbit had been exposed under 1–2 per cent procaine (Merck), 0.2–1 per cent ACh applied locally reduced slightly the amplitude of the slow waves. A reduction in amplitude of the slow waves was also yielded by the application of $1:10^5$ ACh to the motor cortex of the cat under dial. These effects thus resemble those of eserine, although they are much less pronounced.

Effects of ACh on eserinated cortex. The consequences of the application of ACh to the eserinated cortex are similar in the rabbit and cat. The designation of the areas of the rabbit's cortex in the legend of Fig. 2 are according to Rose (1931). Eserine in 1 per cent solution is applied to the cortex and the effects above described are induced (Fig. 2B; 3B). While these responses are continuing a square of 1 per cent ACh is applied to the cortex between the electrodes; within 30 sec.–2 min. large, rapid, rhythmical waves appear (Fig. 2, C; 3, C). Each wave is a complex consisting of two diphasic spikes with smaller components superimposed; the spikes themselves we designate the Es-ACh spikes (Sp.). In Fig. 2 there are 10 complex waves/sec.; the average amplitude of the spikes above and below the isoelectric

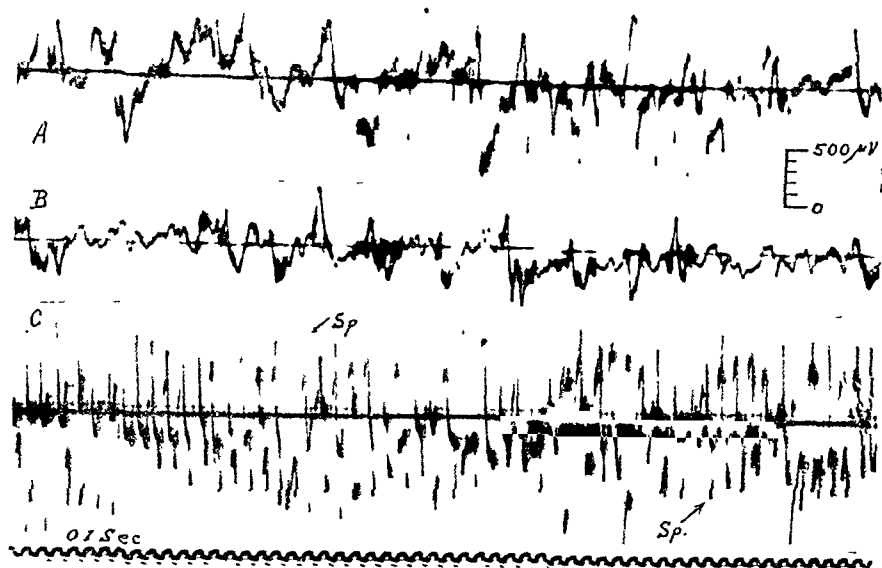


FIG. 2. Effects of eserine and ACh on electrocorticogram. Rabbit. Dial. Electrodes 17 mm. apart; anterior electrode on *regio praecentralis granularis* (Rose), posterior electrode on *area striata* (Rose).

A. Before drugs.

B. 3 min. after application of square of 1 per cent eserine midway between electrodes and painting cortex with small brush of 1 per cent eserine. Reduction in amplitude of slow waves and of large fast waves.

C. 1 min. after application of square of 1 per cent ACh midway between electrodes. Sp., Es-ACh spikes. Vibrissae movements occur. Spikes began 30 sec. after applying ACh.

line in Fig. 2 is $650 \mu\text{V}$. In Fig. 3 there are 8 complex waves/sec.; in this case the average amplitude of the spikes above the isoelectric line is $500 \mu\text{V}$, while the average amplitude of those below the same line is $250 \mu\text{V}$. The spikes resemble in appearance the strychnine-spikes described by Dusser de Barenne and McCulloch (1936).

In another experiment a square of 0.2 per cent ACh was applied midway between the electrodes to the eserinated cortex of a rabbit under dial; in 30 sec. periodic bursts of Es-ACh spikes developed. The first square of 0.2 per cent ACh was then replaced by a second of 0.2 per cent ACh, whereupon the spikes at once appeared continuously and also motor effects, consisting in vibrissae movements.

A matter of importance is the proof that in order to evoke the Es-ACh spikes it is essential that the application of eserine precede that of ACh; the reverse order fails to evoke the spikes. Thus, for their production, ACh must act on the eserinated cortex. Control records from the opposite cortex indicate that the effects of both eserine and ACh are due to local action on the cortex. Indicative of cortical stimulation, motor effects, more intense than those from eserine alone, often accompany the Es-ACh spikes: they are



FIG. 3. Effects of eserine and ACh on electrocorticogram. Cat. Dial. Electrodes 22 mm. apart; anterior electrode on anterior sigmoid gyrus, posterior on suprasylvian gyrus.

A. Before drugs.

B. 4 min. after square of 1 per cent eserine and painting cortex with 1 per cent eserine. Eserine effect.

C. 5 min. after square of 1 per cent ACh on posterior sigmoid gyrus. Sp., Es-ACh spikes.

more evident in the rabbit, in which they consist of vibrissae movements, tremors and mastication; the latter was observed in the rabbit under procaine during the incidence of the Es-ACh spikes; it was due to stimulation of the cortical area of mastication (Ferrier, 1886).

Prevention of Es-ACh spikes by atropine. The influence of atropine was examined in the rabbit by the intravenous injection of 4 mg. (in a rabbit of 3400 g.) and the local application to the cortex of a 0.2 per cent solution of this drug. In this state of complete atropinization the subsequent applications of 1 per cent eserine and 1 per cent ACh fail to evoke the Es-ACh spikes.

Pilocarpine. It was decided to test the effect of another parasympathomimetic drug, namely pilocarpine. The application of 1 per cent pilocarpine to the cerebral cortex of the rabbit does not yield any appreciable changes in the electrocorticogram. Also the subsequent application of 1 per cent ACh fails to evoke waves like the Es-ACh spikes. Thus, pilocarpine is not a sensitizer for ACh as is eserine.

DISCUSSION

When 1 per cent eserine is applied to the cortex it diffuses in small amounts into the cell layers; there, according to evidence presented in an earlier paper (Miller, 1937), it facilitates transmission at cortical synapses, revealing its action by characteristic muscular responses; this facilitation

appears to be of the nature of stimulation of the synaptic junctions. Since muscular responses of the kind mentioned accompany the eserine electrical effects, these latter may themselves be regarded as manifestations of stimulation of cortical synapses by eserine. The synaptic stimulation by eserine may be due to direct action by the drug; however, it will be recalled that ACh evokes, from the (non-eserinized) cortex, effects like those of eserine, namely a slight reduction in amplitude of the slow waves. Hence, we may infer that ACh stimulates synapses; also that the synaptic stimulation by eserine may depend on its known property of inhibiting cholinesterase, with resulting lessened destruction of natural ACh, which would serve as the actual stimulating agent.

We may now consider the electrical effects of the application to the cortex of 1 per cent eserine, assuming it to be an excitant of synapses. The reduction in amplitude of the slow and of the large fast waves may be attributed to the asynchronous firing of large numbers of neurones and this, in turn, may be ascribed to the stimulation by eserine of multitudes of synapses, to widely differing degrees. These differences in synaptic stimulation will depend on the concentration gradient of eserine as it diffuses below the cortical surface; on variations among synapses in susceptibility to the drug; and on numerical differences in synapses in different cortical layers (Lorente de Nó, 1938). In consequence of the above factors asynchronous firing of the synaptically controlled neurones ensues: this is observed as reduction in amplitude of the waves. Conditions are somewhat analogous to those in the optic ganglion of *Dytiscus*, in which faint light abolishes the large waves of the dark rhythm (Adrian, 1937).

ACh when applied to the eserinated cortex, evokes both the Es-ACh spike potentials, as well as the pronounced motor effects described above; both responses may thus be regarded as evidences of cortical stimulation. They illustrate the close interaction generally recognized as existing between eserine and ACh. Reasons have already been given for believing that ACh alone stimulates synapses; thus we may infer that ACh stimulates more intensely synapses previously eserinated; this stimulation being powerful will tend to be of equal intensity at large numbers of synapses and, in consequence, there will result synchronous activity of multitudes of neurones, with the electrical accompaniment of the Es-ACh spikes.

With bipolar recording the spikes above the isoelectric line indicate synchronization of neurones under one electrode, the spikes below the same line synchronization under the other electrode; thus the subelectrode synchronizations are largely out of phase with each other. In Fig. 2 the approximate equality of the spikes above and below the isoelectric line is dependent on the paper square of ACh being placed midway between the electrodes. On the other hand, the greater amplitude of the spikes above the isoelectric line in Fig. 3 is the consequence of the proximity of the ACh square to the anterior electrode.

In the process of neuronal synchronization through synaptic stimula-

tion by eserine and ACh the cells with short axons may be conceived as playing an important rôle for several reasons: each of these cells controls a large number of pyramids and is itself the site of termination of many synapses; further, these cells exist in every cortical layer (Lorente de Nó, 1933; 1938).

That preliminary atropinization prevents the development of the Es-ACh spikes may be interpreted as an instance of the known property of atropine of precluding the effector response by ACh; we would mention also that certain observations, which we have made, indicate as well the possibility of a direct antagonism between eserine and atropine. In general our results with atropine afford substantiation for our views that ACh stimulates eserinizd synapses. The highly specific nature of the actions of eserine and ACh is emphasized further by the failure of pilocarpine to act as sensitizer for ACh.

Our results are of interest in relation to the suggestion of Dale (1934) that pre-existing ACh, or an ACh-like substance, may be concerned normally in transmission through central synapses. In this connection we would offer the hypothesis that, in consequence of the stimulation or facilitation of synapses by eserine and ACh, streams of repetitive electrical impulses are initiated or accelerated across the synaptic junctions; these impulses lead to the discharge of the effector neurones.

SUMMARY

1. The changes in the electrocorticogram in the cat and rabbit, induced by the local application of minimal amounts of 1 per cent eserine, $1:10^5$, 0.2-1 per cent acetylcholine (ACh) and 0.2 per cent atropine, were recorded by one or two cathode ray oscillographs.

2. Application to the cortex of 1 per cent eserine causes a reduction in amplitude of the slow waves and of the large fast waves; these changes indicate cortical stimulation, since they are associated with motor effects.

3. Application to the non-eserinized cortex of $1:10^5$ -1 per cent ACh evokes potential changes comparable to, though weaker than, those from 1 per cent eserine. ACh in 0.2-1 per cent solution applied to the previously eserinizd cortex evokes the Es-ACh spikes; associated motor effects show that the spikes indicate cortical stimulation. For the causation of these effects eserine must precede ACh, the reverse order being ineffective.

4. Atropinization of the animal precludes induction of the Es-ACh spikes and motor effects.

5. Pilocarpine locally is inert towards the electrocorticogram; further, it does not sensitize the cortex for ACh. The drug effects are not due to pH changes.

6. Eserine and ACh are believed to stimulate or facilitate synapses; eserine is a synaptic sensitizer for ACh. The synaptic action of eserine may be partly direct and partly due to its inhibition of cholinesterase, with lessened destruction of effective (natural) ACh. Potential changes from eserine are believed due to unequal stimulation of synapses with consequent

asynchronous firing of neurones. The Es-ACh spikes are believed due to powerful synaptic stimulation with synchronization of many neurones; the cells with short axons may be involved in this synchronization.

7. The hypothesis is proposed that, in consequence of stimulation of synapses by eserine and ACh, repetitive electrical impulses are initiated or accelerated across these junctions.

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CORD POTENTIALS IN SPINAL SHOCK

SINGLE VOLLEYS*

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INTRODUCTION

MUCH has been learned about the electrical activity of the spinal cord by recording from two leads on its surface (10, 11, 12, 13) from two leads on a dorsal or ventral root (1 and 2), from one lead on a root and one on the cord (4 and 1) and from one on two roots and one on an inactive point (25). So extensive is the activity, however, that further analysis requires a more restricted lead. One approach is by the use of microelectrodes, such as those employed by Umrath and Umrath on the cord (24) and by Forbes, Renshaw, and Rempel (6) on the hippocampus. Another is the study of potentials under conditions which limit the spread of activity. We have chosen the latter method and spinal shock as our tool. Or, to express the other aspect of a divided interest, we have employed cord potentials for the analysis of the more or less transient depression of reflexes induced by transection of the cord.

TECHNIQUE

Since our earlier work there have been several changes in technique. A better cathode ray oscillograph and a more efficient amplifier, for the design of which we are indebted to Dr. J. F. Toennies, have facilitated recording; a pair of thyrotrons and transformers proved useful for stimulation; and glass tubes with four silver electrodes in the form of loops have enabled us to lead off action potentials from nerves and roots. In most of our experiments exposure of the cord has been limited to two openings just large enough to permit slitting the dura and placing under it an electrode consisting of a silver disc of not over 2 mm. in diameter. Decerebrate cats and dogs, and young *Macaca mulatta* monkeys, ranging in weight from 3 to 4 kg., have been studied at various intervals after midthoracic section. The monkeys were not decerebrated but had a bilateral ablation of the motor and premotor cortex. Semitendinosus and quadriceps femoris were selected for myographic recording because they suffer less severely than do the muscles of the lower leg from the degenerative nerve changes which complicate reflex recovery in the spinal monkey (3 and 9).

Location of spinal shock. There is ample evidence connecting spinal shock with deprivation of innervation from the brain (20, 21, 22, 19, 7, 8, 9, 14, 15, 16, 17) but none has been offered to determine whether the block be at the internuncials or at the motoneurons. The first alternative should be associated with limitation of the internuncial potential; the second with reflex deficit out of proportion to such reduction. Since occlusion (10, 1), inhibition (12, 13, 2), and facilitation (13) have all been demonstrated in the potentials recorded from the dorsal surface of the cord, it is evident

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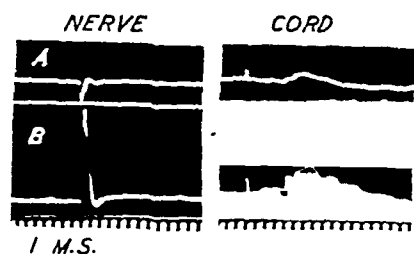


FIG. 1. Latency of cord potential. (A) threshold shock; (B) Maximal shock for A fibers of afferent nerve (sciatic). Note identity of latency. Monkey—6 hours after transection. 4/18/39.

neurones. In contrast to the results of Eccles and Sherrington (5) in the case of the *flexor reflex*, in which an unknown and possibly variable number of links are involved, Lorente de N6 found the value relatively constant and almost unaffected by the strength of the stimulus. Figure 1 illustrates a similar constancy and independence of the number of units involved in the latency of the intermediary potential of the cord. The total latent period is 4.07 msec. whether the stimulus be minimal or maximal for the A fibres of the peroneal nerve. The conduction rate deter-

mined from the time interval between stimulus artefact and nerve potential and the distance from cathode to active lead on the nerve is 75

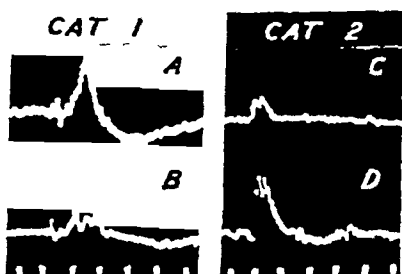


FIG. 3. Effect of transection on cord potentials of cat. (A) decerebrate; (B) 13 min. after transection. 7/20/37; (C) another cat, 6/17/37, 13 min. after transection; (D) 2 hr later.

that the potentials arise from a point of convergence. Since they are not affected by antidromic volleys into the motoneurones (11, 1), their internuncial origin is, in our opinion, firmly established. Further evidence of the post synaptic source of the cord potential is afforded by determination of its latency.

Synaptic Delay. The only experiments of which we are aware in which latency of transmission across a single set of synapses has been analysed, are those of Lorente de N6 (18) upon *oculomotor*



FIG. 2. Cord potential with (A) both leads on cord; (B) Indifferent lead on denervated foot. Monkey 10 days after transection. 5/24/38. Note similarity in positive wave.

meters per second; and the distance to the cord is about 225 mm. Hence only about 3 msec. are occupied in nerve conduction and about one must be attributed to synaptic delay. This estimate agrees with those in three monkeys in which dorsal roots were stimulated. These gave total latencies of 0.75, 1.56 and 1.66 msec. Of these three determinations only the first was in an acutely spinal animal before the onset of histologic changes in the internuncial perikarya. Such values afford additional support to the view that the potentials recorded from the dorsal surface of the cord arise in internuncial neurones.

EFFECT OF TRANSECTION UPON CORD POTENTIALS

Barron and Matthews (1) observed no positivity when leading from dorsal roots in acutely spinal frogs, cats, and monkeys. They regard the positive wave seen when leading from the cord as the result of activity and hence of negativity at the level of the upper lead. We, too, have encountered diphasicity of this type and do not doubt that it is the correct interpretation

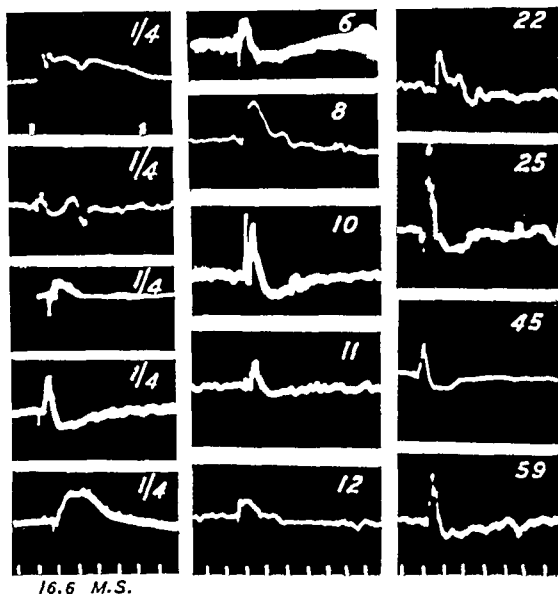


FIG. 4 Cord potentials in response to single volleys maximal for A fibers in 14 *Macaca mulatta* monkeys. Figures refer to interval between transection and recording in days. Note the increase in frequency and extent of positivity with chronicity.

of their data. On the other hand, experiments with the indifferent lead on the foot of a denervated limb have given records of positivity at the active lead indistinguishable from those obtained with the indifferent lead on the cord at the last thoracic or first lumbar level. Such records are shown in Fig. 2. These we regard as evidence of positivity developing within the cord. Because of their interest, experiments showing positivity have been the subject of much discussion. Yet, we cannot emphasize too strongly that when recorded immediately after transection, the animal which shows

positivity is the relatively rare exception. From our own records we can select twelve consecutive experiments on acutely spinal cats without a single case of positivity. In the chronically spinal animal, on the other hand, positivity is the rule. Figure 3 illustrates the extent of reduction of both negative and positive components induced by spinal transection in the decerebrate cat and the degree of recovery observed during the course of an acute experiment. Figure 4 illustrates

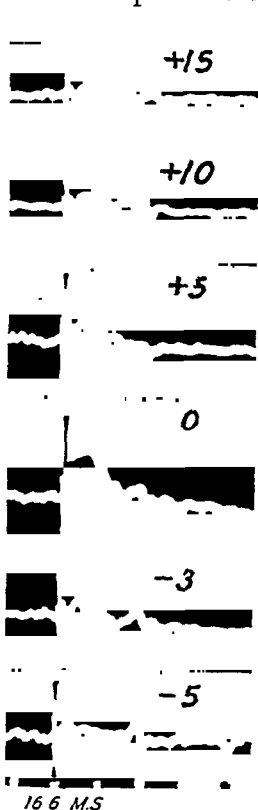


FIG. 5. Spread of cord potential in monkey 12 days after transection. Figures indicate distance in mm. of active lead above (+) and below (-) entrance zone of root stimulated. 2/16/39.

cord potentials induced by single volleys just maximal for A fibres in the peroneal nerve in a series of monkeys at various intervals after transection. The increase in incidence and extent of positivity with chronicity is obvious.

Limitation of region of internuncial activity

In the acutely spinal cat, Hughes and Gasser (11) traced the spread of the intermediary potential 30 mm. above and 10 mm. below the center of the root entrance zone. With this picture the acutely spinal monkey presents a striking contrast. We find that a shift of the lead of three to five millimeters above or below the optimal position may suffice to lose all trace of the potential. With increasing intervals after transection it extends further and further up and down the cord. Figure 5 shows the degree of spread 12 days after operation. Even at this stage of recovery, activity is demonstrable for only fifteen millimeters above and five millimeters below the optimal lead, or half the distance observed by Hughes and Gasser in the acutely spinal cat.

Thresholds. A roughly quantitative method of estimating the degree of shock at each part of the reflex arc is afforded by comparison of the thresholds for the spike potential of the afferent nerve, the intermediary cord potential, and the reflex response. This approach is limited by differences in adequacy of the two leads and by the restriction of myographic recording to a single muscle. We have recorded the cord potential at an amplification of 15 mm. per msec., the nerve potential at one of 3 mm. per msec. Presumably such thresholds represent the activity of several units.

In the cat and the dog transected under divinyl ether anaesthesia, from which recovery occurs a few seconds after withdrawal of the anaesthetic, the threshold of cord potential and reflex are usually identical and distinctly higher than that of the afferent nerve when first recorded. Both values fall progressively for about an hour when they closely approximate or equal that

recorded for the nerve. In this correspondence of threshold for nerve and cord we confirm the earlier result of Toennies (23, p. 380) on the cat. Here again, the monkey contrasts sharply with the cat and the dog. Figure 6 plots against days after transection the ratio of thresholds of potential of cord and afferent nerve and that of reflex and cord potential in a series of monkeys. The first value attains unity within six hours, the second not before twelve days and usually only after a considerably longer period. In the monkey reflex contraction of semitendinosus has never been encountered even to maximal stimulation in an acute experiment. On the other hand, if the lower extremities are left intact and stimuli are applied to a dorsal root, feeble flexion of digits or slight movement at the root of the tail have been observed at values that approximate threshold for nerve and cord in the acute preparation.

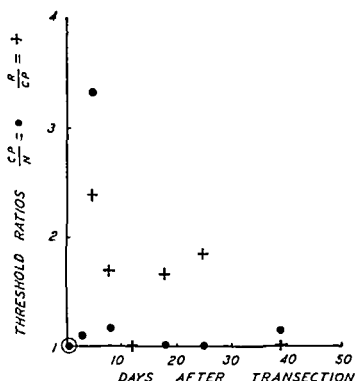


FIG. 6. Ratio of thresholds $\frac{\text{cord potential}}{\text{afferent nerve}}$

= black circles; $\frac{\text{reflex}}{\text{cord potential}}$ = Crosses; plotted against the interval after transection in days.

DISCUSSION

In the cat and the dog there is a period of about an hour after transection during which a minimal afferent volley fails to induce a cord potential. Yet any volley which yields a potential from the cord gives rise to a reflex response. To this rule occasional exceptions are found; but they are as apt to occur in chronic as in acute preparations and hence are probably due to factors other than spinal shock. Both cord potentials and reflexes, continue to change over a far longer period; yet at no interval after transection do they show a lack of parallelism. In these animals the only site at which we have demonstrated spinal shock is at the internuncial level. In the monkey, the restriction of the cord potential is far more severe; yet it appears to be less profound than the reflex depression. The evidence obtained from single volleys suggests that the gulf which separates the spinal monkey from the spinal cat and dog is due in large measure to shock to the motoneurones.

SUMMARY AND CONCLUSIONS

In cats, dogs, and monkeys cord potentials and reflex responses to single afferent volleys have been recorded at intervals after spinal transection ranging from a few seconds to two months.

The latency of the cord potential is independent of the strength of the stimulus and long enough to indicate an internuncial origin.

The effect of transection upon the cord potential is to reduce the amplitude and spread of the negative components and to abolish or almost abolish the positive components. These effects are far more severe and prolonged in the monkey than in the cat or the dog.

In the cat and the dog the threshold for cord potential and ipsilateral flexor reflex sampled in semitendinosus are equal and fall progressively during the first hour after transection at the end of which they approximate that of the afferent nerve. In the monkey, on the other hand, though the cord potential threshold approximates that of the nerve within six hours, the reflex threshold for semitendinosus requires twelve days or longer to attain that level. Reflex responses from this muscle have never been observed even to maximal stimulation in an acutely spinal monkey. On the other hand, stimulation of dorsal roots has given feeble toe flexion and slight tail movement at thresholds approximating those of cord potential and afferent nerve in an acute experiment.

Hence, in the cat and the dog, spinal shock has been demonstrated only at the internuncial level. In the monkey, on the other hand, in addition to more severe internuncial depression, there is evidence of deep and prolonged shock to the motoneurones.

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CORD POTENTIALS IN SPINAL SHOCK

MULTIPLE STIMULI*

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Recovery curves

IN EXPERIMENTS on cats with two volleys over the same afferent fibers Hughes and Gasser (2) correlated two types of recovery curve with characteristic features of the cord potential. When the potential contained only negative components, recovery was rapid enough to be limited solely by

refractoriness; when a positive wave was recorded, it was associated with prolonged reflex unresponsiveness. Figure 1 illustrates the same relation in spinal monkeys. In the acute preparation positivity is almost absent and the cord potential from the second volley has recovered completely in 20 m.sec., a value which agrees well with that obtained by Lorente de Nó and Graham (5) in the case of oculomotor motoneurons in the cat. The presence of a potential of over forty per cent of the height of the control at even the shortest interval is presumably

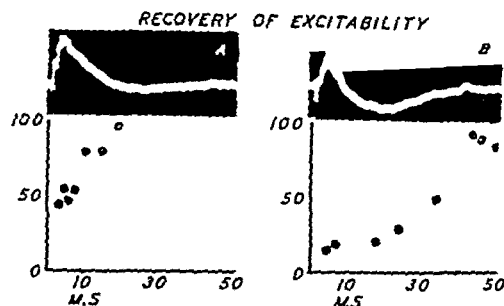


FIG. 1. Relationship of positivity to recovery of cord potential in the spinal monkey. (A) No positivity. Recovery in 20 msec. 6 hours after transection. (B) Positivity present. Recovery in 42 msec. 45 days after transection. 11/29/38-1/5/38.

due to units facilitated by the first volley and discharged by the second as noted by Forbes, Querido, Whitaker and Hurxthal (1) in the case of reflex responses. In the chronic animal the positive wave is well developed and lasts almost to the end of the recovery curve. Depression of the second cord potential is profound and prolonged. At 20 m.sec. (the interval of complete recovery in the acute case) the second cord potential is only twenty per cent of the height of the control. This we regard as inhibition.

Form of tetanic cord potential

Figure 2 illustrates the form of the tetanic potential in the afferent nerve and in the internuncial neurones of the cord, the former at an amplification of 3 mm. per mv, the latter at one of 15 mm. per m.sec. In the nerve

* Aided by a grant from the National Committee for Mental Hygiene from funds granted by the Supreme Council Thirty-third Degree Scottish Rite Masons, for Research in Dementia Praecox.

records this amplification is too low to demonstrate the positive after potential. In the response of the cord to a single volley the positive wave is obvious. At low frequencies, subsequent volleys giving negative waves that

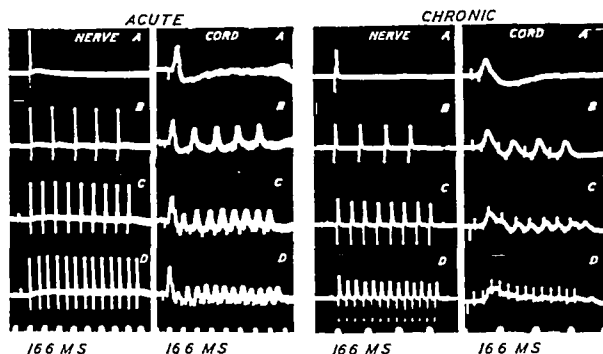


FIG 2 Response of the acute (6 hours) and chronic (45 days) spinal monkey to stimulation of the peroneal nerve at varying frequencies. Stimuli maximal for A fibers. Rates per second: acute monkey (A) single volley (B) 26, (C) 68, (D) 94. Chronic monkey (A) single volley, (B) 77, (C) 142, (D) 232 11/22/38-1/5/39

are but slightly reduced, add little or nothing to the positivity ascribable to the first volley. As the frequency is increased within the range at which there is no reduction of spike height in the afferent, the second internuncial crest is severely cut and those which follow attain a height intermediate between it and the first crest. The positivity, though no deeper than that from a single volley, is prolonged to the end of the tetanus. Finally, at frequencies in which the afferent spikes are slightly reduced by relative refractoriness, the negative internuncial crests are greatly diminished in height, overlap each other, and obliterate any trace of positivity. A comparison of the internuncial responses to a single volley and to a tetanus of over two hundred per second presents a picture so different from the behavior of peripheral nerve as to suggest an origin from perikarya or dendrites. The virtual absence of positive wave at low frequencies in all responses after the first recalls the similar picture previously reported (3) in

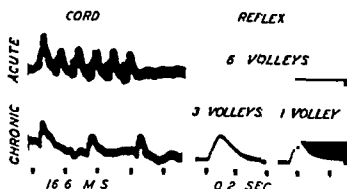


FIG. 3 Comparison of internuncial and reflex activity (semitendinosus) resulting from multiple volleys in the acute (6 hours) and the chronic (59 days) spinal monkey. Peroneal stimulation 1/22/38-1/12/39.

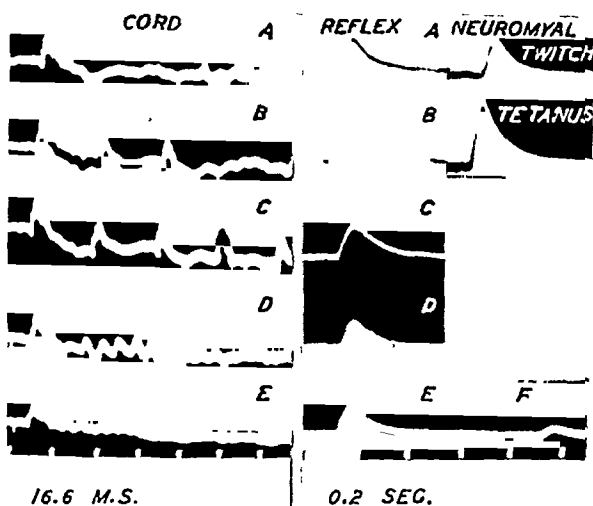


FIG. 4. Cord and reflex response (semitendinosus) in chronic spinal monkey (59 days). Peroneal stimulation (A) single volley, (B) 3 shocks, rate 40 per sec., (C) 5 shocks, 50 per sec., (D) 8 shocks, 150 per sec., (E) 10 shocks, 230 per sec., (F) reflex response to second period of stimulation 0.8 sec. after E. Stimuli maximal for A fibers. 1/12/39.

the face of repetitive stimulation is illustrated in Fig. 3. Even the chronic animal, which gives a large reflex to a single volley, suffers little addition to the response from subsequent volleys (Fig. 3 and 4). Such small reflex increments as these contribute are in inverse relation to the frequency of stimulation. The attribution of these results to the state of the muscle is eliminated by the neuromyal twitch tetanus ratio which contrasts sharply with the reflex picture (Fig. 4). In the reflex record at the bottom of Fig. 4, a second period of stimulation occurs 0.8 second after the first and yields a contraction of only 20 per cent of the tension of the preceding response. Thus, even two months after transection, when the single volley reflex is fully restored, it is elicited at the cost of prolonged succeeding depression. Whether this should be regarded as inhibition or fatigue is difficult to decide. Inhibition is usually observed in association with internuncial positivity and is roughly commensurate therewith in intensity and duration, though frequently outlasting the recorded electrical change. Here, on the contrary, little positivity is added to that from the initial volley, though negative waves are but slightly

the case of crossed inhibition of a single volley and supports the view then advanced that negativity and positivity result from activity of different units.

Reflex shock

So far as the intermediary potentials are concerned, the effect of repetitive firing is similar in the acute and the chronic monkey as illustrated in Fig. 2. Figures 3 and 4 illustrate the contrast between the internuncial activity and the reflex depression. In the preceding paper it was stated that a reflex response of semitendinosus to a single volley in the monkey had never been observed. Similar silence in

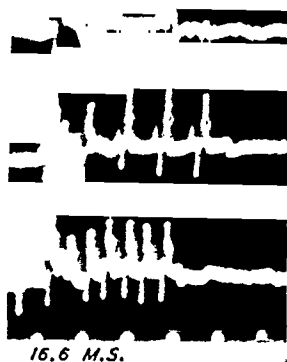


FIG. 5. Cord potentials of acute cat. 5/23/39.

reduced at frequencies yielding a tetanic tension scarcely exceeding that from a single stimulus. When it is recalled that in the cat tetanic stimulation gives an internuncial response indistinguishable from that of the monkey (Fig. 5), but a reflex tetanic tension twice that from a single volley (4), it is difficult to resist the conclusion that the difference in reflex response is due to differences in the state of the motoneurons rather than in the nerve impulses playing upon them. In so far as this opinion depends upon comparison of cat and monkey, however, it hinges upon the assumption that inhibition of the reflex may be measured at the internuncial level as accurately in the latter animal as in the former.

So far as ipsilateral inhibition is concerned, this appears to be true (Fig. 6). Hence we incline to the view that in even the chronic spinal monkey there persists, at a point downstream from the internuncials whose activity is recorded, a significant degree of depression.

SUMMARY

In the spinal *Macaca mulatta* monkey, inhibition of the internuncial response to the second of two volleys to an afferent nerve is contingent upon the presence of a positive wave in the preceding cord potential. In the absence of such positivity, internuncial recovery from refractoriness is complete in 20 to 25 msec. Repetitive stimulation gives a series of internuncial potentials in which little positivity is added to that incident to the initial volley. Such a pattern contrasts sharply with that obtained from peripheral nerve and hence suggests a perikaryal origin and different units for the sources of negativity and positivity respectively.

The acutely spinal monkey gives no contraction of semitendinosus in response to afferent stimulation by either single or multiple volleys. The chronic monkey yields a large contraction to single volleys to which little is added by further stimulation even at frequencies at which the cord potentials are but slightly reduced. The view is advanced that in even the chronic monkey there persists a considerable degree of depression downstream from the internuncials recorded, presumably at the motoneurons.

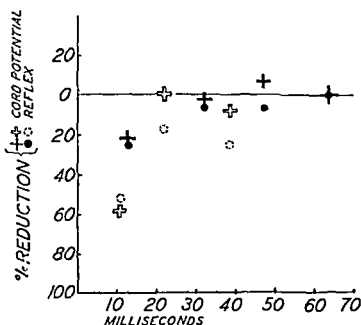


FIG. 6. Recovery curve of ipsilateral inhibition of flexor reflex (semitendinosus) and cord potentials. Data from 2 chronic monkeys. Circles refer to reflex, crosses to cord potential. Black symbols, monkey transected 25 days, 7/5/38; white symbols, monkeys transected 59 days, 1/12/39.

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CORD POTENTIALS IN SPINAL SHOCK

CROSSED EFFECTS IN MONKEY, *MACACA MULATTA**

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INTRODUCTION

AMONG the latest reflexes to recover from spinal shock are those that depend upon the efficacy of internuncial neurones that cross from one side of the cord to the other. The localization of points of obstruction in these arcs consequently requires the use of chronically spinal animals whose cords have suffered as little deterioration as possible. So severe is the course of "isolation alteration" in the spinal monkey that only the exceptional preparation is suitable. Consequently, the data presented in this paper lack confirmation upon an extended series. Yet they impress us as sufficiently significant to justify our conclusions.

Recovery of crossed potential

By placing two alternative ground leads as far laterally as is consistent with contact with the dorsal columns it is possible in the acute monkey to demonstrate almost complete restriction of the cord potential to the side of stimulation (Fig 1A). In an animal recorded twelve days after transection (B) the contralateral potential is still far smaller than the ipsilateral, but at fifty nine days (C) the height is the same on both sides of the cord. Row D illustrates potentials from a monkey whose cord was hemisectioned on the right sixty four days and transected three days before recording. Stimulation of a nerve of the acute side gives rise to a considerable ipsilateral and a brief contralateral potential. Stimulation on the chronic side presents a picture complicated by spread of activity to the upper lead which reduces the magnitude of the potential recorded from the chronic side and reverses the sign of the potential recorded from the crossed lead. The brevity of the negative potentials and apparent increase in contralateral positivity in the chronic spinal monkey (C) are presumably due to the same diphasic artefact. This is indicated by the records of the bottom row, E, taken from an acutely spinal cat with the grid lead on the foot of a denervated leg. Here the contralateral potential is virtually identical in size and form with the ipsilateral. Such prompt recovery in the cat contrasts sharply with the picture in the monkey even twelve days after transection. The positive wave in the cat potentials emphasizes a point insisted upon in the first paper of this series: that spinal positivity is by no means always a diphasic artefact.

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To return to the recovery of the crossed cord potential; that of the contralateral side attains equality with the ipsilateral in a few hours in the cat, within two months in the monkey.

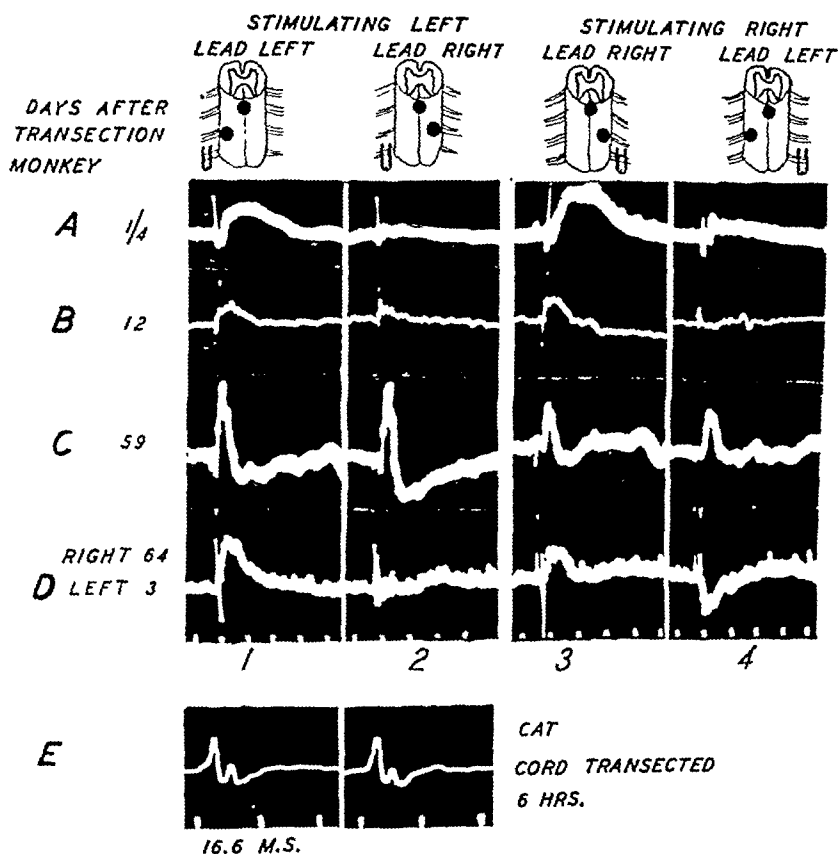


FIG. 1. Ipsilateral and crossed cord potentials recorded with lower lateral ground and upper medial grid lead at various intervals after spinal transection.

Column 1 Stimulus left, lead left.

Column 2 Stimulus left, lead right.

Column 3 Stimulus right, lead right.

Column 4 Stimulus right, lead left.

A, C, and E, stimuli to peroneal nerves.

B and D, Stimuli to 7th lumbar dorsal roots.

Note complete recovery of crossed potential in monkey (C) after 59 days, in cat (E) after 6 hours. A 11/29/38; B 2/16/39; C 1/12/39; D 2/7/39; E 5/23/39.

Source of crossed cord potential and site of its block. These questions may be advantageously approached by chronic hemisection followed by acute transection in the monkey. Records from such a preparation are shown in Fig. 2. Several of these are marred by artefacts from the electrocardiogram (the downward deflections in A2, B1 and 2, C2, and D1) which may be ignored. On stimulation of a dorsal root of the chronic side, there is a well

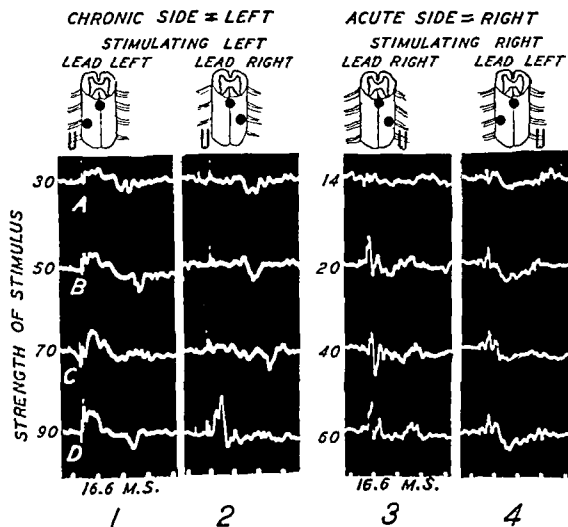


FIG 2 Ipsilateral and crossed cord potentials recorded with lower lateral ground and upper medial grid lead in response to various strengths of stimulus to the 7th lumbar dorsal roots in a monkey hemisected on the left 39 days, transected 5 days before recording. Note the difference in threshold of the crossed potential of the two sides. Downward deflections in A2, B1 and 2, C2 and D1 are artefacts from the electrocardiogram 3/7/39

developed ipsilateral potential at a strength of 30 (arbitrary units) but no contralateral potential even at 70. At 90 a contralateral potential appears after a latency of 6.4 msec. or over 5.6 msec. longer than that on the side of stimulation. On the other hand, when a root of the acute side is stimulated, the potentials of both sides appear at the same threshold of 14 and with stronger stimulation the amplitude of the contralateral potential is not far short of that of the ipsilateral.

Since the thresholds for both potentials are the same in response to a root of the acute side, it is evident that when a root of the chronic side is stimulated, the crossing neurones must be transmitting impulses in response to stimulation far below

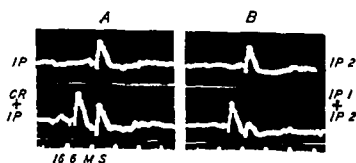


FIG 3 Absence of crossed inhibition (A), contrasted with marked ipsilateral inhibition (B), of cord potential in a monkey 59 days after transection. Mid dorsal leads. Stimulation of peroneal nerves 1/12/39

threshold for the crossed potential. Since there is no reason we know of why the potentials of the crossing axones should not be as readily recorded

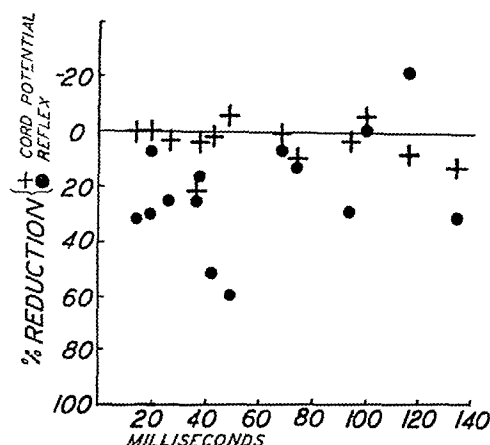


FIG. 4. Recovery curve of crossed inhibition of the flexor reflex (semitendinosus) in the chronic monkey recorded in Fig. 3. Note the frequent occurrence of reflex inhibition without reduction of the negative cord potential. Stimuli maximal for A fibers of peroneal nerves. 1/12/39.

McCouch and Stewart (4) and Bonnet and Bremer (2), have advanced evidence for the occurrence of inhibition at the internuncial level in the cat. Even in the cat, however, crossed inhibition of the ipsilateral flexor reflex is usually slightly more profound than that of its cord potential, although the corresponding recovery curves parallel each other to a remarkable degree (4, p. 413). In the monkey, we have recorded crossed inhibition in only a single animal, which had been transected fifty-nine days previously. The crossed cord potential was fully developed (Fig. 1C). Ipsilateral inhibition at the internuncial level was profound (Fig. 3). Crossed inhibition, though not sufficiently consistent to give a smooth curve (Fig. 4) was occasionally definite in the myographic record. Yet the corresponding cord potential developed to almost or quite the full height of the control (Fig. 5). Hence, in the monkey, crossed inhibition may occur down-

as those of axones of the dorsal horn cells on which they terminate, we are forced to the conclusion that the contralateral cord potential is associated with activity of perikarya or dendrites sufficient in magnitude or duration to yield a far larger potential record than that due to spike potentials of axones in their vicinity. The same data lead to another conclusion: that the block of the crossed potential results from a raised threshold of the cells of the contralateral dorsal horn and not from lack of impulses playing upon them. Finally, they afford further conclusive evidence of the internuncial origin of cord potentials.

Site of crossed inhibition of ipsilateral flexor reflex. Lorente de N  (5), Hughes and Gasser (3), Hughes,

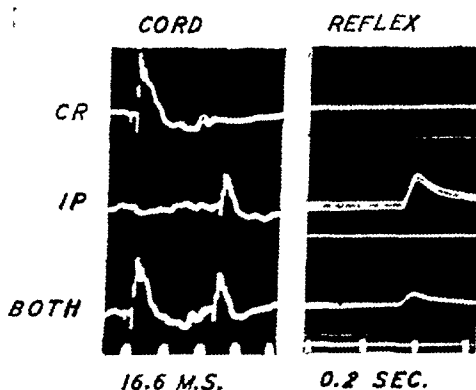


FIG. 5. An example of the contralateral reflex inhibition without reduction of cord potential plotted in Fig. 4. 1/12/39.

stream from the internuncials recorded by the cord potential, presumably at the motoneurons

SUMMARY

In the spinal *Macaca mulatta* monkey the contralateral component of the intermediary cord potential is scarcely detectable in the acute preparation, is small at 12 days after transection but has attained the magnitude of the ipsilateral component within two months

In the chronically hemisectioned, acutely transected animal, the ipsilateral potential spreads farther up the cord when the stimulus is applied to afferents of the chronic side than when to those of the acute side. When the chronic side is stimulated, the threshold for the crossed cord potential is far higher and its latency far longer than that for the potential on the side of stimulation. When afferents of the acute side are stimulated, the thresholds for ipsilateral and contralateral potentials may be identical and the latter potential may approximate the magnitude of the former. From these data the following conclusions are drawn:

1 The contralateral cord potential is associated with the activity of perikarya or dendrites and not merely with that of axones

2 Block of the crossed cord potential is due to a high threshold of the cells of the contralateral dorsal horn and not to lack of impulses playing upon them

3 The above data yield evidence of the internuncial origin of the cord potential

In the cat crossed inhibition of the internuncial potential parallels that of the flexor reflex, though usually it is somewhat less intense. In the chronic monkey, on the other hand, crossed inhibition of the reflex may occur without reduction of the corresponding cord potential. This indicates a locus of inhibition downstream from the internuncials recorded—presumably at the cells of the anterior horn

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AN ATTEMPT TO PRODUCE SLEEP BY DIENCEPHALIC STIMULATION*

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CONSIDERATION of the literature indicates that at present there are two main theories of sleep, with many modifications of the parent theories. One theory proposes that sleep is a state of diminished activity of the brain and is a passive process; the other suggests that sleep is an active inhibition of the cortex and lower centers by a so-called "sleep center." Since electric currents can be used for either stimulation or destruction of living tissues by a proper choice of wave form and intensity, it seemed possible that stimulating or destructive electric currents could be used in the brain to obtain data bearing on the mechanism of sleep.

Many workers have used electric currents in experiments involving large areas of the nervous system, but few experimenters have attempted to investigate small areas where the site of action of the current was accurately known. Fewer still have controlled the experiments by preparing histological sections and examining the tissues for visible damage. Hermann (1885) placed marine animals in water through which a current was flowing and the animals aligned their bodies in the direction of current flow ("galvanotropism"). Scheminsky (1936) reported that when the alignment was such that the head was toward the anode the animal was very quiet or in a state of "electronarcosis." Scheminsky explained the depression as a state of anelectrotonus of cell bodies. However a state of electronarcosis has been produced with alternating currents in marine forms (Rosenberg, 1928, and Harreveld, 1937) and in mammals (Sack and Koch, 1933). Tschagowetz (1912) found that "galvanonarcosis" could be produced in cats by putting an anode on the head and a cathode on the sternum. Respiration and pulse rate were normal during the period of narcosis. The results with cats could not be repeated on dogs by Ivy and Barry (1932).

Direct current has been used locally in the brain by Marinesco, Sager and Kreindler (1929). Anodal polarization in the tubero-infundibular region facilitated sleep, whereas cathodal polarization caused excitement. The effective region was between the optic chiasma and mammillary bodies, between the junction of the thalamus and hypothalamus and between planes just lateral to the columns of the fornix on each side. Currents through the thalamus or caudate nucleus seemed to have no effect.

Electric stunning of animals has been performed by many workers with

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the type of current used by Leduc (1907). This was a unidirectional interrupted current with a square wave form at about 100 cycles per sec. and an interval to impulse ratio of 1 to 9. Leduc applied the current through external electrodes, the cathode being placed on the head of the animal and the anode in the midline of the back. The direction of current flow was opposite to that in the electronarcosis experiments of Scheminsky and others.

Pachon and Delmas-Marsalet (1924) employed a feeble galvanic current interrupted 20 to 30 times per sec. to stimulate the caudate nucleus of dogs in the waking state. Chewing, licking and swallowing movements and grimaces occurred, and at times there were circus movements to the side opposite the one stimulated. Somnolence was not produced; and if the animals were asleep they were awakened by such a stimulus. Hess (1932) advocated a unidirectional current interrupted 4 to 15 times per second with the rate of rise and fall of current retarded by self induction coils and condensers. During work on peripheral nerves, Hess found that use of a current of this type resulted in approximation of the thresholds for fibers in autonomic and somatic nerves, and therefore advocated its use in the interior of the brain. Hess used a unique method for orientating electrodes in the brain and checked the approximate position of the electrode tips after each experiment. His methods were an improvement over the free hand methods of previous workers, although they were probably not as accurate as is possible with the use of the Horsley-Clarke machine followed by microscopical control of every experiment.

The method of approach in the present experiments was to pass electric currents through localized regions in the brain of an unanesthetized animal. If stimulation of a point caused sleep, the results could be explained as stimulation of a "sleep center" which actively inhibited other parts of the brain. If, however, destruction of an area produced sleep it would indicate that a decrease in activity of the brain permitted sleep and that sleep was a passive process. In this latter case the region involved when functioning normally could be thought of as a "waking center."

METHODS

Cats were used in all experiments. Under ether anesthesia a Horsley-Clarke machine was attached to the animal's head, a threaded iron tube was screwed into a trephine hole in the skull and electrodes lowered into the brain. The electrodes and use of the Horsley-Clarke machine have already been described (Ranson, 1934). By means of a special electrode holder carried by the stereotaxic instrument, one to three pairs of electrodes could be inserted at a time. Usually one pair was used with an electrode 3 mm. on either side of the midline. When more than one pair was used, one pair was 1.5 to 3 mm. rostral to the other. The electrodes were cemented in place with dental investment compound. The Horsley-Clarke machine was then removed, the external parts of the electrodes were cut short and scraped, and ether anesthesia discontinued. The animal was injected with 50 cc. of physiological saline intraperitoneally and left for four to five hours to recover from the ether. The cat was next tested as to the state of its pupils, posture, respiration, response to pain, motor initiative, righting reactions, placing reactions, perseverance of positions, resistance to passive movements, gait and emotional expression in the presence of a barking dog in order that subsequent changes might be noted. Two 30 gauge silk covered wires about 4 feet long connected the electrodes to the current source and allowed the animal to move without restraint.

Four different current sources were used. The first was simply a battery, series resistor and milliammeter for steady direct current. The second was of the type used by Hess with a mercury interrupter, and coils and condensers to modify the rate of change of current. The wiring diagram was the same as Fig. 12b, page 23, of Hess' monograph (1932). The addition of inductors and condensers of high values permitted the retention of the interruption of current and depolarization as specified by Hess but allowed elimination of the stimulating factor. The capacitance could be made as high as 85 microfarads and the inductance as high as 69 henries. This was more than enough to prevent stimulation of any

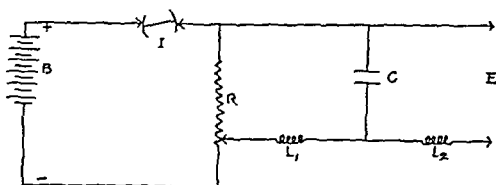


FIG. 1. Stimulating circuit. B, 12 volt battery; I, interrupter; R, 27 ohm slide wire; C, 4 microfarad condenser; L_1 and L_2 , 1,700 ohm air cored coils; E, wires to milliammeter and animal.

peripheral or central nervous structure that was tested. The third current source contained a mechanical interrupter and was arranged as shown in Fig. 1. The resistance of the slide wire was negligible compared to the resistance of the coils and tissues. The condenser was the same value as recommended by Hess and the total resistance of the air cored coils was nearly the same. Although we were unable to find out the inductance of his coils, we checked the wave form of the current with a cathode ray oscillograph and found it to correspond to that in his figures. Measurement of photographic records of the oscillographic trace revealed that the current reached 95 per cent of its full value in 16 msec. The interrupter was set so that current flowed for one-fifth of each cycle with 5 interruptions per sec. The fourth current source was a Harvard inductorium. The four sources gave us then a steady direct current for the production of lesions, an interrupted direct current for the production of lesions, an interrupted direct current for stimulation and a faradic current for stimulation.

In a typical experiment, an animal that had recovered from anesthesia was thoroughly tested. The current being used was then passed through the brain for 30 sec. unless some reaction occurred. After the current was turned off the animal was watched for delayed effects and then examined as to gait, posture, etc., just as at the start. The current was always weak at first and was gradually increased in subsequent 30 sec. periods until an intensity was reached that changed the behavior of the animal.

Immediately after the final observations, sometimes following the procedures described above and sometimes 12 to 24 hours later, the cat was anesthetized with ether, bled through the inferior vena cava and the brain injected with 10 per cent formalin through the carotid arteries. The electrodes were removed and the brain prepared for histological sectioning. The area of the brain penetrated by the electrodes was sectioned serially at 50 micra and every fourth section stained by the method of Weil. Adjacent sections were stained with cresyl violet. The relations of the electrodes to fiber tracts and nuclei and damage done to those structures were studied microscopically. An attempt was then made to correlate the physiological results of the experiments with the structures stimulated or destroyed.

RESULTS

The results obtained in our experiments permit division of the cats into five groups: (1) somnolent, (2) somnolent with a loss of motor initiative when awakened, (3) cataleptic, (4) excited and (5) negative. Cats that did not appear normal after operation and those in which histological study revealed incorrectly placed electrodes or damage to the brain were discarded. Fifty-four experiments were considered valid and will be reported here.

Somnolent cats. This group of seven cats includes only those which showed somnolence without exhibiting plasticity and loss of initiative when awakened. Animals displaying the latter signs, whether somnolent or not, were grouped with the cataleptic cats. Of these cats, three were treated

with steady direct current and four with a non-stimulating interrupted direct current. Somnolence was not produced by a stimulating current. A representative cat was number 35 which reacted as follows:

A non-stimulating interrupted direct current with a peak value of 0.4 mA. was applied for a total of one minute. The animal appeared to be somnolent but was awakened by slight pulling of hair over the abdomen and arose to an erect position. Ten minutes later the drowsiness had disappeared. Another minute of current caused the animal to go to sleep again in a normal position and this time the drowsiness lasted an hour. Throughout the somnolent period the animal could be easily awakened and when aroused demonstrated normal motor control and initiative, but if left alone would curl up and appear to sleep. The animal recovered from this last period of somnolence and seemed normal in every respect.

Microscopic examination of the brain disclosed that lesions had been produced at the tips of the electrodes (Fig. 2). The lesion on the left side was in the lateral hypothalamic area rostral to the mammillary nuclei. It lay between the anterior column of the fornix and the cerebral peduncle but damaged neither. The lateral hypothalamic area was markedly damaged, but the zona incerta, nucleus of the field of Forel and the subthalamic nucleus were spared. The lesion on the right side was also in the lateral hypothalamic area but spared cells rostrally and caudally. About half the fornix was demyelinated as was half of the mammillothalamic tract.

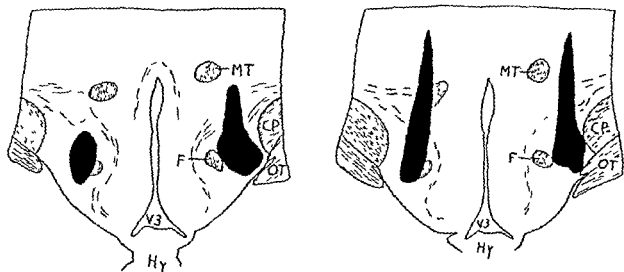


FIG. 2. Diagram of two sections through the hypothalamus of cat 35. Lesions are in solid black. F, Fornix; Hy, Hypophysis; CP, Cerebral Peduncle; OT, Optic Tract; SN, Substantia Nigra; V3, Third Ventricle; MT, Mammillothalamic Tract.

Five animals of this group had the tips of the electrodes placed bilaterally in the lateral hypothalamic area just rostral to the mammillary nuclei. Two had electrodes placed in the lateral group of nuclei of the thalamus, but the current required to produce somnolence was greater and of longer duration (e.g., 3 mA. for 3 minutes in cat 4) and the degree and duration of the somnolence was less than in the hypothalamic experiments.

Cats with loss of motor initiative. Animals in this group exhibited somnolence and when awakened showed subnormal motor initiative. They would stand or lie for many minutes without initiating voluntary movements and responded with a minimum of movement to loud noises and handling. However, these cats could not be molded into abnormal positions as can be done with truly cataleptic cats.

Of the eight cats in this series, five had electrodes placed in the lateral hypothalamic area and three in the thalamus. The current used in two of the hypothalamic cats and all experiments on the thalamus was a steady direct current, the strength of which was about 1 mA. in the hypothalamic experiments and as high as 3 mA. in the thalamic experiments. The current used in the other hypothalamic experiments was a non-stimulating interrupted direct current with a peak value of 0.4 mA. Upon histological study, the damage found in the hypothalamic experiments was similar to that in the previous group. There was no constant difference in the lesions of the first two groups to explain the loss of initiative in one group and not the other. Cat 14 can be described as typical of the thalamic experiments.

Cat 14 was treated with a steady direct current of 3 mA. for 3 one minute periods before becoming drowsy but was definitely somnolent at the end of the fourth period. The animal was very easily aroused by noises or touching and reacted vigorously to pinching of its tail. Abnormal postures were quickly righted and there were no signs of plasticity. The gait was normal and righting reflexes were active, but placing reactions were absent. There seemed to be a loss of initiative as the cat would remain in one place if left alone, frequently going to sleep.

Examination of the brain disclosed extensive bilateral thalamic destruction. On the left side the lesion destroyed almost the entire lateral group of nuclei and extended from the level of the rostral border of the posterior commissure to the anterior group of thalamic nuclei. There was considerable damage to the lateral habenular nucleus, pulvinar, dorsomedial, nucleus, nucleus centralis lateralis and some to the paracentral nucleus. There was a little involvement of the nucleus ventralis pars anterior. The lesion on the right side was more laterally placed but had the same rostro-caudal extent as that on the left.

Cataleptic cats. This group includes seven cats that exhibited somnolence, plasticity and loss of motor initiative, and includes three cats with spasticity and loss of motor initiative which did not become somnolent. The signs were similar to those described by Ranson and Ingram (1932) for cats with retromammillary lesions. Examples of this group are cats 31 and 32.

Cat 31 was treated with a non-stimulating interrupted direct current of 0.4 mA. peak value for a total of 2 minutes. Slight somnolence was produced, but the most marked changes were the development of a high grade plasticity and a total loss of motor initiative. The cat was indifferent to handling and would maintain any position into which it was put. The lesions were bilateral in the lateral hypothalamic area dorsolateral to the mammillary nuclei as shown in Fig. 3.

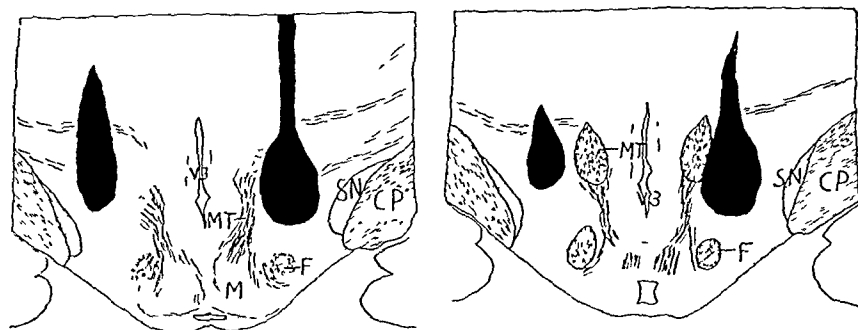


FIG. 3. Diagram of two sections through the hypothalamus of cat 31. Lesions are in solid black. Labels as in Fig. 2.

Cat 32 developed plasticity and loss of initiative but did not become somnolent. The type of current was similar to that of cat 31 but the lesions were a little more ventrally placed as is indicated in Fig. 4.

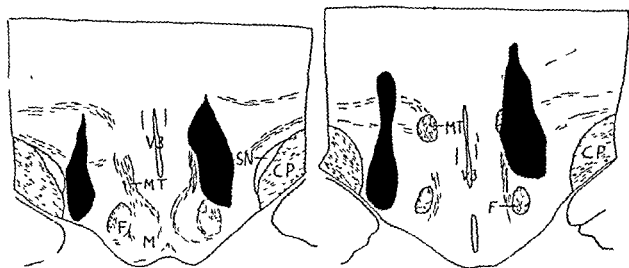


FIG. 4. Diagram of two sections through the hypothalamus of cat 32. Lesions are in solid black. Labels as in Fig. 2.

All 10 cats of the cataleptic group had lesions placed bilaterally in the lateral hypothalamic area and extending into the posterior hypothalamus. The current used in all of this group was of a destructive type. Plasticity and somnolence were not produced by a stimulating type of current.

Excited cats. Twenty-one cats treated with an interrupted stimulating direct current exhibited excitement, but none of them became somnolent. In many of the experiments faradic stimulation was carried out with little change in the type of response. Electrodes were placed in various parts of the thalamus, in nearly every part of the hypothalamus, the junction of the thalamus and hypothalamus and near the anterior commissure.

Stimulation of the hypothalamus in a waking animal resulted in responses previously described for the anesthetized animal, such as pupillodilation, respiratory changes, etc. In addition the cat usually became restless, walked around looking from side to side and cried softly, but rarely became markedly agitated if the current was kept low. Stimulation of the *thalamus* in the waking animal has been described by Kabat, Anson, Magoun and Ranson (1935) and Mussen (1934) so need not be repeated here. Lapping movements were produced by stimulation just ventral to the anterior commissure.

Negative cats. Eight cats reacted very little to passage of current through the brain. In four of these the heads of the caudate nuclei were destroyed bilaterally by a 3 mA. direct current. A transient drowsiness may have developed in one of these but could not be repeated. Cats 5 and 17 had very extensive destruction of the caudate nuclei following 9 minutes of a 3 mA. current with no signs of drowsiness, loss of initiative, plasticity, forced movements or loss of placing reactions. A destructive current was used in the hypothalamus of one cat and in the thalamus of two cats with little or

no response. Stimulation of the septum pellucidum in one cat and just ventral to the anterior commissure in two others yielded little of importance.

DISCUSSION

The results indicate that when somnolence is produced by passage of an electric current through the diencephalon, the somnolence is due to destruction and depression and not to stimulation. None of the cats treated with a stimulating type of current became somnolent. Where the somnolence was transient, it can be assumed that the lesions seen microscopically were not solely responsible for the drowsiness. In the present experiments involving the hypothalamus, animals exhibiting slight or transient signs usually had small lesions. Other animals with lesions intentionally made large remained comatose until sacrificed. It is likely that animals with small visible lesions really had larger functional lesions at the time of deepest somnolence.

Electrostatic and electrolytic effects of currents can certainly depress nervous tissue even when not severe enough to produce histological changes. Any direct current of sufficient intensity to stimulate the interior of the brain will have a concomitant destructive action varying with the duration and intensity of the pulses. To decrease the rate of change of current with coils and condensers will increase the threshold of the tissues. A higher strength of current is then needed with its greater powers of depression. The argument that the effects of current spread can be eliminated by such a modified current has been discussed by Harrison, Magoun and Ranson (1938) and does not justify the use of such a current in the interior of the brain.

Clinically the hypersomnia due to pathology of the diencephalon has been explained as evidence of a loss of function. Many cases of tumors involving or impinging on the hypothalamus have been reported and are usually associated with somnolence. Study of these cases leaves little basis for the explanation that somnolence is the result of an irritative phenomenon. In the present experiments somnolence was obtained by passing currents through the thalamus and hypothalamus. To produce drowsiness with electrodes in the thalamus, currents of long duration and fairly high intensity were required. When contrasted with the relatively short and weak currents used in the hypothalamus it leaves some doubt as to whether the current used in the thalamic experiments acted on the thalamus alone. From the very size of the thalamic lesions it is evident that the current was not local in action.

However, there is considerable support in the literature for the inclusion of the thalamus in any hypothesized sleep-waking mechanism. Trömner (1928) believes the thalamus is the only structure anatomically suited for a sleep center and suggests that it influences hypothalamic centers. Rowe (1935) discusses a cortico-thalamico-periventricular-hypothalamico-mesencephalic chain which when broken may result in sleep. Ranson (1939)

indicates that part of the hypothalamic drive to the cortex may be by way of the thalamus. Hirsch (1924) and Spiegel and Inaba (1927) give evidence that thalamic damage results in hypersomnia. Much of the action of the current in the present experiments must have been on the thalamus, but the high intensities used permit the criticism of current spread.

In the hypothalamus the visible lesions were in many cases small and discrete. Somnolence was easily produced when the electrodes were in the

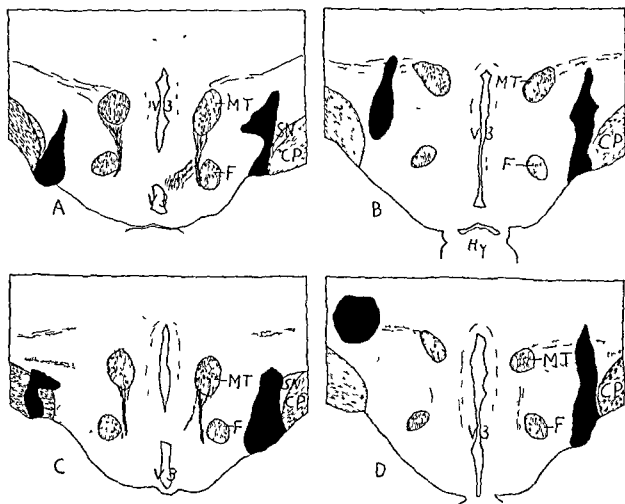


FIG. 5. A. and B, Drawings of two sections through the hypothalamus of cat 8 for comparison and C and D, drawings from sections of cat 10. Lesions are in solid black. Labels as in Fig. 2.

lateral hypothalamic area just rostral to the mammillary bodies. Lesions more medially and caudally placed were usually associated with catalepsy in addition to the somnolence. Difficulty in separation of the site of action of the current, however, is indicated by study of the brains of cats 8 and 10. The electrodes of these two cats were in very similar positions (Fig. 5); cat 8 became somnolent whereas cat 10 became cataleptic.

Stimulation of the various parts of the diencephalon from the posterior part to the anterior commissure resulted only in excitement and increased activity. Since Hess (1932) reported that in reality the microscopical controls in a large number of cases exhibited changes in the region of the stimulus, it is possible that his cases of somnolence may be explained by a temporary or permanent loss of function from electrostatic and electrolytic

effects. This view is shared by Serota (1939) who believes that sleep is associated with a decreased activity of the hypothalamus. Ranson (1939) gives evidence that the hypothalamus functions as a "waking center" when active, but when thrown out of function permits the decreased nervous activity characteristic of sleep. Kleitman in a long series of papers presents the view that sleep is a state of cerebral inactivity following a decrease of afferent impulses. His review (1929) discusses this problem.

Gagel (1936) reported a case of Foerster's in which mechanical stimulation of the posterior part of the hypothalamus produced sleep. Penfield's (1929) case of diencephalic autonomic epilepsy which was attributed to irritation, however, had signs of widespread autonomic discharge rather than somnolence. There seems on the whole to be a paucity of evidence supporting the theory that sleep is the result of an active process. The usual argument that sleep must be due to hyperactivity of the parasympathetic system because of the concomitant miosis, fall in blood pressure, fall in temperature, etc., is not necessarily valid. These are all signs of decreased sympathetic activity and are to be expected when the hypothalamus becomes less active during sleep. Some of the difficulties in explaining the mechanism of sleep have arisen because many workers fail to distinguish between processes leading to sleep and those resulting from sleep. Many of the so-called concomitants of sleep do not appear until after sleep has begun. The correct sequence of events has been studied by Kleitman and Doktorsky (1933) and by Cooperman (1936). Denny-Brown (1932) discusses the problem of active inhibition and states that there is little support for such a theory.

SUMMARY

1. Destructive and stimulating electric currents were applied to the hypothalamus, thalamus, junction of the hypothalamus and thalamus, head of the caudate nucleus, septum pellucidum and region of the anterior commissure in waking cats.

2. Somnolence was produced by destructive action of currents when applied to the lateral hypothalamic area. Catalepsy tended to develop when the lesions involved the medial and caudal parts of the hypothalamus.

3. Somnolence was produced by destructive electric currents when applied to the lateral group of thalamic nuclei, but the strength and duration of the current was much greater than that in the hypothalamic experiments. It is possible that the effects were not solely thalamic.

4. Extensive lesions of the head of the caudate nucleus did not cause somnolence.

5. Stimulation of the structures mentioned above did not yield somnolence.

6. No evidence was obtained to support the theory that sleep is a phenomenon of active inhibition.

I wish to acknowledge the guidance, advice and helpful criticism of Dr. S. W. Ranson throughout the course of these experiments.

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RELAYED IMPULSES IN ASCENDING BRANCHES OF DORSAL ROOT FIBERS

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ONE of the most important questions in connection with the reflex discharge from the spinal cord by way of the dorsal roots is, what fibers carry the impulses? Certain facts only are known. On histological evidence the fibers must have their cells of origin in the dorsal root ganglia. Fibers conducting impulses at all the velocities characteristic for A fibers are involved (Toennies), and a large fraction of the dorsal root fibers are accessible to the discharge. For example, Toennies found that under special experimental conditions it was possible to cause about 35 per cent of the alpha fibers to be occupied by reflexly evoked impulses.

Additional evidence establishing the mediation of the dorsal root reflex by fibers originating from dorsal root ganglion cells will be brought forward in the present paper. Most, if not all, of the large afferent fibers, such as those which contribute to the first large elevation in the action potential of an afferent nerve, are known to divide soon after their entrance into the spinal cord into a short descending branch and a long ascending branch. Many of the ascending branches pass up the dorsal columns to end eventually in the nuclei of Goll and Burdach. Both branches send collaterals into the central grey matter of the spinal cord.

If fibers which divide in this way carry the reflex, it should follow that at the same time that the reflex discharge passes outward toward the periphery, impulses should also be conducted along the central branch toward the nuclei in the medulla.

METHOD

Cats were anesthetized with dial and the cord was exposed. The usual arrangement was to place stimulating electrodes on the sixth or the seventh lumbar dorsal root, and to lead the response by means of a needle electrode placed in the homolateral dorsal column some distance cranial to the site of entrance of the root stimulated. The differential input circuit described by Toennies (1938b) was employed. One of the grids of the input amplifier was connected to the needle electrode, and the other to the adjacent vertebra or muscle. After the electrodes had been put into place, the cord surface was flooded with paraffin oil to prevent drying. Measurements of the rectal temperatures were made, and in about one-half of the experiments the cord temperature was determined with a thermocouple designed for the purpose.

RESULTS

Form of the response in the cord. In the experiment illustrated in Fig. 1, the needle lead was placed in the dorsal column a few tenths of a millimeter below the surface and 13 cm. above the point of entrance of the stimulated

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dorsal root (L 7). When a single afferent volley was initiated by a shock applied to the root, the potential picture shown in the first record of Fig. 1 was obtained. The form of the response is characteristic. The action potential starts with a sharp spike-like elevation and is continued 2.9 msec. later by a second and more prolonged elevation. It can easily be shown that the first spike is the record of the impulses passing directly upward along the central branches of the primary neurons; and no comment need be made about it, beyond calling attention to the amount of the temporal dispersion of the impulses that has been occasioned by the long distance of conduction. The interest centers around the second elevation. If it is to be demonstrated as representing the centrally directed counterpart of the reflex discharge to the periphery, it must be proven to have properties in common with the latter. Experiments were designed to test these properties and their outcome is described in the following paragraphs.

Relaying of the impulses. Without proof at the outset that the impulses originate from activity set up in the grey matter of the cord, there would be no occasion to inquire further. This proof, however, can be obtained in a simple manner. Toennies demonstrated that the reflex is readily inhibited by previous activity. From this observation it should follow that the starting of impulses along the tracts in the dorsal columns should be inhibited in a parallel fashion.

Record C in Fig. 1 shows the effect on the response of an afferent volley produced by the response of a similar afferent volley 13 msec. earlier. The nature of the effect can best be understood by comparing the conditioned response with the control isolated response in record B, immediately above it. All the second elevation has disappeared, leaving in pure form the spike-like potential derived from the ascending primary impulses. Thus, like the reflex impulses in the roots, the second elevation is inhibited. And as the time course of the inhibition is the same in the two, the presumption



FIG. 1. Response in the homolateral dorsal column to a stimulus applied to the seventh lumbar dorsal root. Record C was obtained when the responses to single shocks as shown in records A and B were elicited during the same sweep. The needle lead was 13 cm. cranial to the entrance of the stimulated root. Rectal temperature, 36°C. Time in msec.

is strong that the two are produced in the cord in the same way.

Localization of the response in the dorsal columns. The second point requiring proof was that the second elevation actually represents impulses passing through the dorsal columns. In order to test this point, systematic explorations were made of the regions in the cross-section of the cord from which the response could be led. The cord was examined with the aid of a needle electrode attached to a calibrated microdrive permitting insertion of the needle into the cord at any latitude or depth. Various cord levels were examined in this way, and in every experiment it was found that the potential could be picked up from the homolateral dorsal column; but that as soon as the needle penetrated below the column or crossed the midline, the potential disappeared.

Furthermore it was possible to make out considerable localization within the column, despite whatever lack of precision there may be in the power of the needle leads to localize responses. In the experiment illustrated in Fig. 2 the seventh lumbar dorsal root was stimulated, and a section of the cord was explored 8 cm. cranial to the root entrance. At the conclusion of the experiment the part of the cord from which the potentials had been led was fixed in 10 per cent formalin. Serial sections were subsequently cut, and examined to check the depth and position of the needle tracks. In the figure the positions of the needle at which the potential records were taken are marked on a microphotograph of an adjoining section.

It is clear from Fig. 2 that there is considerable variation at the several positions in the size of the potentials recorded, both with respect to the primary spike and the relayed discharge. A careful examination of the records brings out the fact that the maximum of the activity is along a diagonal line starting laterally at the surface in the region of position 2a and passing medially and ventrally between 2b and 3b, and between 2c and 3c toward 3d. Deep in the white matter the potentials tend to disappear, and near the midline they are very small. The finding thus is nicely in accord with what is known from degeneration experiments about the position of the central projections of the fibers of a dorsal root in the dorsal columns.

A point of greater significance that is brought out in the records of Fig. 2 is the close correspondence as to size between the primary spike and the relayed discharge. The fibers that carry the discharge must have the same position in the columns as do the fibers that carry the primary impulses from the roots. Just as the dorsal root reflex is largest in the fibers of the root which produced it (Toennies, 1938a), the relayed discharge is largest in the tract of the dorsal columns which carries the central projections of the root fibers evoking the discharge.

Latency of the relayed discharge. If the relayed discharge is conducted through branches of the dorsal root fibers that carry the reflex, the latencies of the two events—attributable to the sum of the synapse times in the grey matter of the cord—should be the same. The latency of the second elevation in the record presented in Fig. 1 was 2.9 msec. No figures on the normal

synapse time of the reflex were available for comparison, inasmuch as Toennes' figure of 4 msec was obtained on preparations at a subnormal temperature. In order to supply the information, experiments were undertaken on decerebrated cats in which the cord temperature was maintained at 38.5°C. Stimulating electrodes were placed on one fasciculus of the seventh lumbar

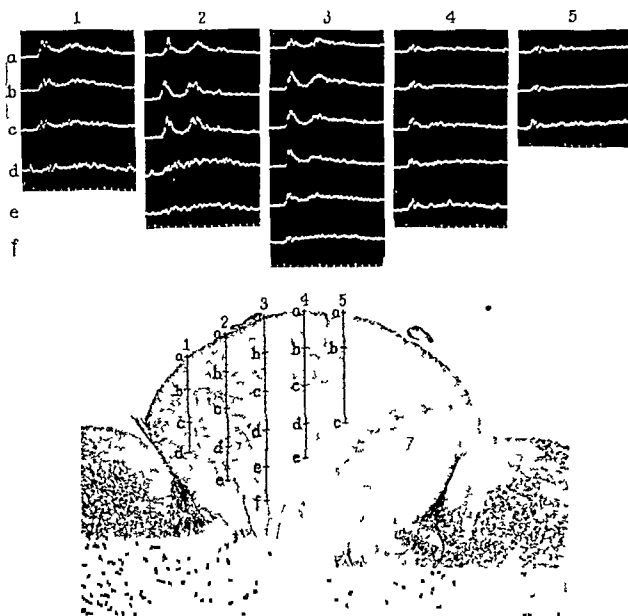


FIG. 2. Localization of the response in the dorsal columns. Stimulus applied to the seventh lumbar dorsal root. The needle leads were 8 cm. cranial to the root at the positions in the dorsal column marked on the microphotograph of an adjacent cord section. Rectal temperature 36°C. Time in msec.

dorsal root, and the recording electrodes on another branch of the same root. After correction for root conduction, the reflex times were found to vary between 2.1 and 2.6 msec. The latency of 2.9 msec for the second elevation in Fig. 1 is thus somewhat outside of the normal range for the reflex, but it was a proper value for the reflex in that particular experiment, as the cord temperature was about 35°C.

Conduction velocity of the relayed discharge. If the relayed discharge is

conducted through the ascending branches of the primary fibers, the velocity of conduction should be the same as that of the primary impulses, subject to the one reservation that if a single fiber carries both sets of impulses, the conductivity must have had time to return to normal between the primary and the secondary occupation. As a corollary of this line of reasoning it

should follow that the second elevation should succeed the primary spike by an interval that is independent of the level of the cord at which the interval is determined.

Experimentation has shown that the interval is constant within the range of experimental error. Figure 3 shows the records from an experiment in which leads were taken at four distances from the root. In order to aid in the identification of the time of start of the second elevation, records were also taken of the primary spike stripped of the relayed impulses which overtake its slower components. The isolation of the spike was accomplished through inhibition of the relayed impulses by a previous response (lower records). When isolated, the spike reveals the base line from which the potentials of the relayed responses rise.

The intervals between the start of the spike and the start of the second elevation were found in the records of Fig. 3 to be the

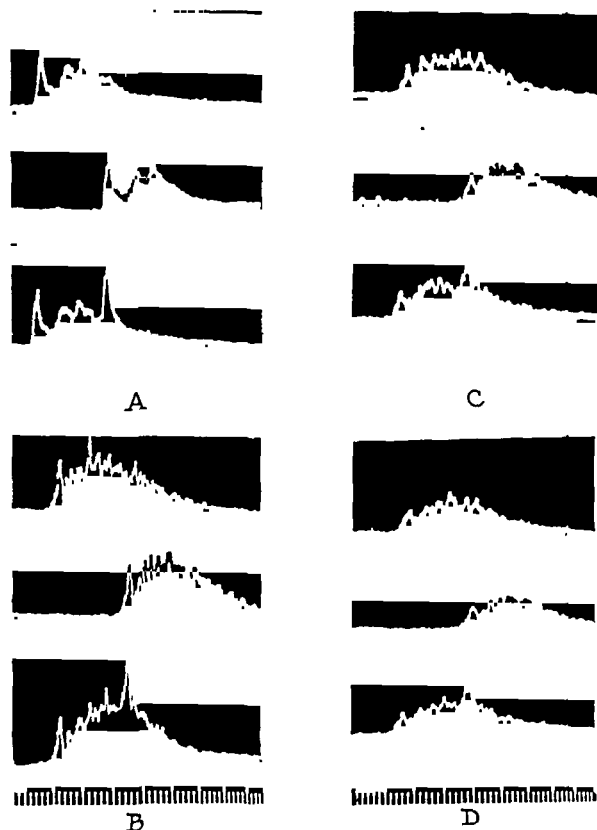


FIG. 3. Action potentials in the dorsal column at four distances of conduction. The stimulus was applied to the seventh lumbar dorsal root, and leads were made with a needle electrode placed successively at various distances cranial to the point of entrance of the root: 5.2 cm., record A; 14.3 cm., record B; 16 cm., record C; 18 cm., record D. Rectal temperature, 32.5°C. Cord temperature, 31.5°C. Time in msec.

following: After 5.2 cm. of conduction, 3 msec.; after 14.3 cm., 3.5 msec.; after 16 cm., 3.0 msec.; and after 18 cm., 2.8 msec. The constancy of the periods indicates that the fastest of the relayed impulses must have the same velocity as the fastest of the directly conducted impulses. At the same time, the fact that the intervals did not get longer as conduction proceeded served to eliminate the possibility that the impulses at the start of the sec-

and elevation might be carried upward in a series of short relays through the fasciculus proprius. The negation of the latter possibility is in accord with the findings in the localization experiments previously cited, as the maximum of the potential was found to be at a distance from the position of the fibers of the fasciculus.

Stimulation of the ascending branches of the dorsal root fibers. Thus far it has been shown that the second elevation in the action potential, recorded from the dorsal columns following the entrance of a single afferent volley into the cord by one of the dorsal roots, is attributable to impulses that have been relayed in the grey matter of the spinal cord and are passing up in the columns toward the medulla without further relay and at a velocity corresponding to that of the primary volley. It now remains to be shown that the impulses are actually conducted in branches of the fibers which carry the dorsal root reflex.

For descriptive purposes the dorsal root fibers may be divided into reflex-producing and reflex-carrying fibers, without prejudice as to whether the two groups are the same or different. A shock applied to the dorsal columns would stimulate the ascending branches of the two groups indiscriminately, and impulses would pass backward over the branches to the bifurcation of the root fibers, and then outward over the roots. At the same time by way of the collaterals into the grey matter of the cord, dorsal root reflexes would be produced, and relayed discharges would be dispatched cranially along the dorsal columns. If the pathways for the latter are the central branches of the reflex-carrying fibers in the roots, the impulses should meet and block any descending impulses in those fibers set up by a second shock applied to the columns; and there would then be a deficit in the number of impulses conducted over the dorsal root fibers, as compared with the number conducted after a control first shock.

After stating in advance that the impulses are blocked, as the theory demands, the evidence will be presented in terms of a specific experiment, illustrated in Fig. 4 and 5. Leads were placed on the seventh lumbar root,

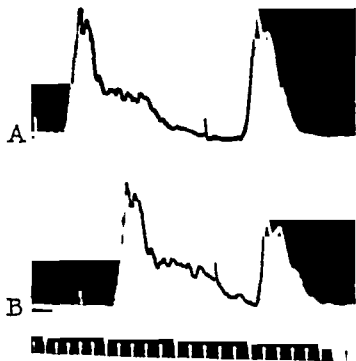


FIG. 4. The response led from the seventh lumbar dorsal root when two stimulating shocks were applied to the homolateral dorsal column 18.5 cm. cranial to the root. The intervals between the shocks were 12.9 msec. (record A) and 9.7 msec. (record B). Rectal temperature, 35°C. Cord temperature, 33.5°C. Time in msec.

with the ground electrode between the cord and the two grid leads, and the root was crushed near the distal grid lead. The stimulating electrodes, which were two small needles separated by 2 mm. and insulated except at the tips, were placed in the dorsal column near the midline, 18.5 cm. cranial to the root.

Two records taken at shock intervals of 12.9 and 9.7 msec. are shown respectively in records A and B of Fig. 4. In both records the first elevation starts with the directly conducted impulses and is continued by the reflex discharge, while the second elevation contains only the directly conducted

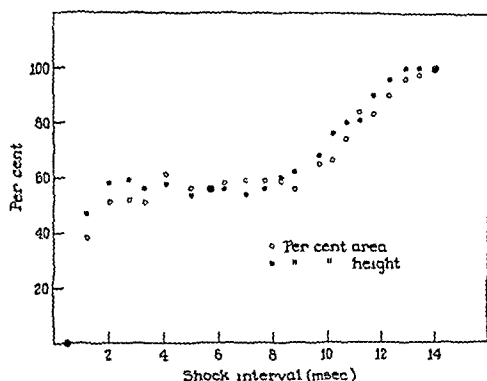


FIG. 5. The percentage decreases in the heights and in the areas of the action-potentials of the impulses set up by a shock applied to the dorsal column and conducted directly to the seventh dorsal root. The decreases are conditioned by the activity set up by a shock preceding the test shock by various intervals. Stimulus and leads as in Fig. 4.

of the slowly conducted impulses that contribute to the tail of the action potential. Between 14 msec. and about 9 msec. the responses decrease progressively. Then they reach a plateau which is maintained until about 2 msec., at which time the responses begin to decrease rapidly.

The explanation of the course taken by the curve is the following. The shortest conduction time between the stimulating electrodes and the root was 2.3 msec., and about 3 msec. would elapse before the first relayed responses would start to pass upward along the column fibers. The impulses ascending from the root level would require a minimum of 2.3 msec. to get back to the locus of the stimulating electrode, and a minimal refractory period of 1.2 msec. (by test) would need to elapse after their arrival before the fibers could be stimulated. The sum of these times is 8.8 msec.

At times longer than 8.8 msec. the fibers having conduction velocities slower than the maximum are blocked. At 8.8 msec. the blockade reaches a maximum, and a shortening of the interval thereafter succeeds only in altering the point along the fiber path at which occlusion takes place. The

impulses set up by the second shock, as the reflex at this time is inhibited. At the longer interval, 12.9 msec., there is no decrease in the height of the second response; but at the shorter interval, 9.7 msec., the height has been reduced 32 per cent.

A summary of the findings for stimulation intervals up to 14 msec. is presented in Fig. 5. The data are given in two ways: The percentage reductions in the heights and in the areas of the responses, as compared with the values at 14 msec.,—at which time the heights had fully recovered. The areas fall off somewhat more rapidly than do the heights, because the area method of measurement takes fuller account

final decrease at very short intervals is occasioned by the refractory period of the column fibers at the stimulating electrodes, following the first stimulus.

DISCUSSION

The sum of the evidence now available indicates that an afferent volley, whether artificially excited or made up of physiologically selected sensory impulses (Toennies, 1939), after a central reflex time of 2.1 to 2.6 msec. evokes a reflex in dorsal root fibers. At the same time that the reflex discharge passes outward to the periphery, the discharge is also carried along the central branches of the dorsal root fibers mediating the reflex toward the nuclei of Goll and Burdach. The centrally directed impulses are conducted along the dorsal columns at the same maximal velocity as that of the directly conducted sensory impulses; and for a given root they occupy the same band of fibers in the dorsal columns as do the sensory impulses. There is no fact that sets off the reflex-carrying dorsal root fibers from those conducting sensory messages. On the other hand, all the facts would be accounted for by the postulate of two fiber systems having similar properties, one system carrying sensory impulses and exciting the reflex, and the other discharging reflex impulses to the periphery and centrally directed impulses toward the nuclei in the medulla. Toennies (1939) has recently shown that the reflexly discharged impulses are able to condition the setting up of sensory impulses at the terminals of sensory fibers. But here again the experiments are susceptible of two interpretations,—an effect on the endings resulting from involvement of the fibers in the reflex, or a modification of the terminals through a postulated but unidentified set of special fibers not carrying sensory impulses. For the second alternative to hold, however, an added reservation has to be made as the result of the findings reported in the present paper. As the reflex-carrying fibers conduct impulses toward the medulla, the expectation would be that they would produce some effect there. If the anticipated effect exists, the only way in which the reflex-carrying fibers could fail to give account of conditions in the periphery would be for their peripheral terminations to be inexcitable.

CONCLUSION

At the same time that a reflexly evoked discharge of impulses passes over dorsal root fibers to the periphery, the impulses are also conducted along the ascending branches of these fibers in the dorsal columns of the spinal cord toward the nuclei in the medulla.

SUMMARY

This investigation was designed to describe in further detail the properties of the dorsal root reflex response recently reported by Toennies. Since the dorsal root fibers divide and send long branches up the dorsal columns to the nuclei of Goll and Burdach, it should follow that simultaneous with the passage of the reflex response outward toward the periphery, a relayed

impulse should likewise pass up the dorsal columns. Cats were anesthetized with dial and the cord was exposed. Stimulating electrodes were placed on the sixth or seventh lumbar dorsal root, and a recording needle electrode was placed in the homolateral dorsal column some distance cranial to the site of the stimulated root. In accord with the expectation a relayed response was recorded following the direct afferent volley. The properties of the relayed response were found to be similar to the properties of the dorsal root reflex. Evidence was accumulated to show that the relayed response was carried by the ascending branches of the dorsal root fibers.

The author takes pleasure in expressing his gratitude to Doctor Gasser for encouragement and advice in the pursuit of this work.

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OBSERVATIONS UPON DIAPHRAGMATIC SENSATION*

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THE phrenic nerve has been of interest to the embryologist, physiologist and surgeon mainly because it supplies a somatic motor innervation to the striated muscle in the diaphragm. The significance of the sensory components of the phrenic nerve was appreciated by Bourgery (1845) and Luschka (1853 and 1863), who was aware of shoulder-tip reference on stimulation of receptors in the phrenic distribution. Among others, Schreiber (1883) observed increases in blood pressure on stimulation of the central end of the cut phrenic nerve in the dog. Greene (1935) reported that dilatation of coronary blood vessels occurred subsequent to stimulation of the central end of the cut phrenic. Likewise, Thornton (1937) found that the phrenic nerve provides at least one afferent path for reflex broncho dilatation, the efferent path of which is in the vagus. Little and McSwiney (1938) have used the pupillo dilator reflex as an index of afferent impulses. In the cat, they found that the afferent fibers in the phrenic nerves enter via the dorsal roots of the 5th and 6th cervical segments and form the main pathway for sensory impulses from the diaphragm. Hinsey, Hare and Phillips (1939) degenerated the somatic motor and sympathetic fibers in the phrenic nerve of the cat and demonstrated histologically that this nerve contains myelinated sensory fibers of different sizes and unmyelinated ones.

That stimulation or irritation of the central portion of the diaphragmatic pleura or peritoneum may cause a pain which is referred into the shoulder tip area is a well-recognized clinical phenomenon in man (Luschka 1863, Ross 1888, Felix 1922, Capps and Coleman 1932, Morley 1931, Woollard, Roberts and Carmichael 1932). This type of reference has been given a number of explanations, one of which was put to test in the experimental animal by Pollock and Davis (1935). Their work indicated to them that viscerocutaneous reflexes caused changes in the environment of the skin in the shoulder tip area. This vasomotor or chemical process was thought to be the result of impulses in sympathetic fibers and in turn to be the cause of stimulation of somatic receptors in the area, thus explaining the reference. However, in 7 of their cases after bilateral removal of the stellate ganglia, pain persisted when the diaphragmatic peritoneum was faradically stimulated. The explanation given was that some connection with the cord still existed. These experiments seemed inconclusive. In the light of the

* This work was presented before the American Physiological Society on April 24, 1937 and is reported in abstract in *Amer J Physiol* 119:336, 1937.

importance which has been attached to them, we have repeated certain phases of them and performed additional experiments.

METHOD

A study was made of nociceptive sensation in the central portion of the diaphragmatic peritoneum of cats and dogs. The spinal cord was completely transected at T4 or higher in all experiments. By breaking ascending conduction from levels below the section, conscious appreciation of sensation from the rim of the diaphragm was not present because this portion receives a sensory innervation from the lower intercostal nerves. Section of the spinal cord at C7 would not only serve to break ascending conduction but would also interrupt descending conduction for the activation of preganglionic neurons in the upper thoracic segments. After the spinal cord had been transected, a midline incision was made through the anterior body wall from the xiphoid process caudally about 8 to 10 cm., from which point the incision was extended laterally at right angles on both sides. By proper retraction of the rostral 2 flaps of body wall, it was possible to expose the abdominal surface of the diaphragm and apply to it bipolar faradic stimulation of rather high intensities (Harvard inductorium, 1 dry cell, coil separation 6-8 cm.). Consciousness of nociceptive stimulation in the lightly anesthetized or unanesthetized animals was indicated by vocalization, protrusion of claws, movements of the vibrissae, dilatation of the pupils, hyperpnea, and struggling. In our experiments, the pupils dilated when the diaphragm was stimulated after all possible thoracolumbar sympathetic pathways to them were interrupted, *i.e.*, transection of spinal cord at C7 or bilateral removal of sympathetic chains from above superior cervical ganglia to below the 7th thoracic chain ganglia. These were demonstrations of the significance of inhibition of the parasympathetic innervation in the dilatation of the pupil occurring subsequent to nociceptive stimulation (See Ury and Gellhorn, 1939).

At the termination of each experiment, a careful autopsy was performed to control the operative procedures.

OBSERVATIONS

Faradic stimulation of the central portion of the diaphragm induced consciousness of nociceptive sensation in all cases after the following experimental procedures were carried out:

1. The vagi and sympathetic trunks were sectioned bilaterally in the neck below the superior cervical sympathetic ganglion and the spinal cord was transected at C7 (four cats). Observations were made within 24 hours.

2. The right and left sympathetic chains were removed from above the stellate through T7 and the spinal cord was transected at T4 (1 cat). This animal was observed during the subsequent 24 hours.

3. The right and left sympathetic chains were removed from above the stellates through T6; the right and left vagi were cut high within the thorax; the spinal cord was transected at T3. This cat was observed the following day.

4. The right and left sympathetic chains were removed from above the superior cervical ganglia to below the 8th thoracic ganglia. After 22 and 26 days respectively in 2 animals, the spinal cord was transected at T4.

5. Cat No. 59.

Jan. 15, 1937. Removed right and left superior cervical sympathetic ganglia and the sympathetic chains in the neck down through the middle cervical sympathetic ganglia.

Feb. 5, 1937. Removed right and left thoracic sympathetic chains from above stellate ganglia to below T7.

Feb. 26, 1937. Cut ventral roots of left C3-4-5-6-7 spinal nerves.

March 15, 1937. Spinal cord transected at T3 and peritoneal surface of diaphragm stimulated.

6. The spinal cord was transected at C7 in 3 dogs.

7. The spinal cord was transected at C7 in 2 dogs in which both stellate ganglia had been excised and in which absence of regeneration into the cervical sympathetic trunk was proven by functional tests (stimulation of isolated 1st, 2nd, and 3rd thoracic ventral roots).

8. In one cat, both vago-sympathetic trunks were sectioned in the neck caudal to the superior cervical sympathetic ganglia; the spinal cord was transected at C7; both phrenics were sectioned above the diaphragm (artificial respiration was instituted). Stimulation of the proximal portions of each phrenic nerve elicited nociceptive responses.

The experiments leave no doubt that the sympathetic pathways to the head and to the cervical nerves can be broken in their peripheral distribution and that descending conduction in the spinal cord to all the segments supplying preganglionic fibers can be interrupted and still there can be consciousness of nociceptive sensibility in both the cat and dog on stimulation of the central portion of the diaphragmatic peritoneum. When the margin of the diaphragm (within 5 mm. of its attachment to the body wall) was stimulated, an ipsilateral tetanus of the muscle of the diaphragm resulted but there were no nociceptive responses. On the other hand, in all of our experiments, stimulation of the central area was accompanied by nociceptive responses. However in this area, particularly in the tendinous portion, there were points which were silent and did not respond. These were present in animals in which the only operative procedure was transection of the spinal cord at the 3rd and 4th T level and thus are not attributable to an absence of sympathetic pathways. The distribution of receptors in the various portions of the diaphragm serves as the most likely explanation for these silent areas. In experimental procedures which involve the fixation of electrodes in the peritoneal surface of the diaphragm, an absence of response may possibly be due to a stimulation of one of these insensitive areas.

DISCUSSION

The sensation produced by stimulation of the central portion of the diaphragm is subsequent to afferent conduction over sensory fibers in the phrenic nerve and ascending pathways in the central nervous system to higher centers. This sort of work in the experimental animal is limited by the fact that we have to rely upon certain signs in an absence of a description of sensation. From these experiments, we cannot conclude that viscerocutaneous reflexes play no part in the nociceptive sensation produced by stimulation of the central portion of the diaphragmatic peritoneum. It does seem justifiable to state that it has been demonstrated that responses associated with nociceptive sensation in the experimental animal can occur

when viscerocutaneous reflexes are experimentally excluded from participation. A crucial experiment in man would consist of stimulation of the central portion of the diaphragmatic peritoneum or pleura in a case where there has been transection of the spinal cord at the lower cervical level. This information could be obtained by a similar stimulation in a case in which stellectomy had been performed to relieve peripheral vascular disease. It would be profitable to study shoulder tip pain in phrenectomies where the incision could be enlarged so as to expose the stellate ganglion which could be blocked with novocaine.

In the work of Weiss and Davis (1928), complete relief of referred pain was obtained by the injection of novocaine to produce skin anesthesia in the area of reference in 21 of 25 cases. Their series did not include any cases of shoulder-tip pain. Morley (1931) infiltrated the right shoulder area with novocaine in 2 cases of shoulder-tip reference in perforated duodenums. The spontaneous referred pain was abolished in one and reduced in the other. Under spinal anesthesia, the abdomens were opened. Stimulation of the dome of the diaphragm on the right side caused a shoulder pain in both cases but it was less severe than when the left side was stimulated. Woollard, Roberts and Carmichael (1932) mapped the area where pain was referred on stimulation of the central end of the cut phrenic in 9 cases where this nerve was avulsed for therapeutic reasons. The area belonged to the distribution of the 4th cervical nerve, was about the size of a shilling, was situated internal to the acromio-clavicular joint, and was almost identical in all the subjects they examined. When this area was infiltrated with novocaine, they reported that the anesthesia of the area in no way affected the character or intensity of the referred pain which with the exception of an occasional slight shift in the locality of the reference remained the same.

Doctor W. K. Livingston of Portland, Oregon, has very kindly prepared a brief of a case attended by Doctor Theodore Adams of that city. With their permission, the following interesting observations made by Doctor Livingston will be quoted concerning this case of a subphrenic abscess in a woman 30 years of age:

"On Oct. 22nd she developed severe right abdominal pain, associated with pain in the right flank and at the tip of the shoulder. A subphrenic abscess was drained. The fluid was serous rather than purulent at that time.

"On Nov. 15th because of evidence that there was a residual accumulation of pus present, a much larger incision was made after resection of the distal portion of the 11th rib, and a large purulent abscess was encountered under the dome of the diaphragm. Drains were left in for some time.

"On Dec. 18th I passed a uterine probe through the incision to explore over the dome of the liver for any possible residual pockets of pus. I found that when the dome of the diaphragm was touched with the probe she complained of pain in the right shoulder. The pain occurred instantaneously with the contact of the probe against the diaphragm. Drawing the probe back a half inch and manipulating it so as to stimulate the skin around the incision failed to elicit complaint of pain. The pain in the shoulder immediately recurred when the probe again touched the diaphragm even though the patient was not aware that the probe was being moved. The pain was ascribed to a well-localized area just medial to the junction of the clavicle and the acromion process. Very slight shifting of the position of the probe against the dome of the diaphragm seemed to alter the location of the

pain, at least the patient did not always indicate exactly the same spot on the shoulder, but she never once failed to complain of pain the instant the diaphragm was touched.

"In order to determine whether the pain in the shoulder was due to some physico-chemical change in the skin affecting pain receptors of the cervical nerves, or was due to some deeper and more direct mechanism, I infiltrated the skin with novocaine in the area to which the pain was ascribed. One percent novocaine was used to raise a wheal in the skin some three inches in diameter, with its center at the acromio-clavicular juncture. She again complained of pain in the shoulder the instant the diaphragm was stimulated and the area to which the pain was referred did not seem to be altered.

"On the possibility that I had not infiltrated a wide enough area of skin to rule out the explanation of the 'referred pain' advanced by Pollock and Davis, two days later I repeated the experiment. This time the novocaine infiltration of the skin was extended to cover the entire supra-clavicular fossa, the top of the shoulder and well down over the deltoid and both front and back of the shoulder surface until the wheal was six or seven inches in diameter. Never once did the patient fail to report pain in the shoulder when the diaphragm was touched and although she seemed a little less able to localize the pain exactly, on several instances she pointed to the center of the novocainized area to which the pain had been originally referred."

These observations of Livingston's and those of Woollard, Roberts and Carmichael (1932) make it evident that pain can be referred into the shoulder-tip area when its skin is anesthetized. This would rule out chemical changes in the skin as the cause of referred pain. Morley's (1931) suggestion that unblocked nerves from the 3rd and 4th segments might explain residual sensation after infiltration of the skin seems inadequate. Livingston's injections were made in such a manner that a very large area was involved and it does not seem likely that there could have been unblocked nerves to the skin in this area. These negative observations do not rule out necessarily the positive ones of Weiss and Davis (1928) because in certain areas referred pain might be abolished by local injections and in others it might not be. Furthermore, if a local infiltration did relieve referred pain in an area, it would not prove that there had been a local chemical change induced by impulses in autonomic nerves. If afferent impulses from the viscera were facilitated by the normal flow of sensory ones from somatic areas, removal of the latter by local block might render the visceral inflow inadequate to cross the threshold of consciousness or might reduce the quantum of sensation experienced there.

The animal experiments reported here in both the cat and dog demonstrate conclusively that the thoracolumbar sympathetic pathways are not essential for nociceptive sensation from the central portion of the diaphragm. Whatever takes place must be looked for in the spinal cord and central nervous system. Morley's (1937) assumption of antidromic dorsal root impulses being responsible for peripheral referred pain is not justifiable on evidence available at present. It has been shown that impulses coursing over visceral afferent fibers may inhibit or facilitate muscular reflexes (Dusser de Barenne and Ward 1937, Kaufman 1938, Schweitzer and Wright 1937). Likewise, visceral afferent impulses may very well change the threshold in the spinal cord to afferent pain impulses from the periphery by virtue of their terminating upon the same groups of neurons.

The "hypersensitive" or hyperirritable focus concept of Mackenzie

(1921) and others takes on real meaning if we assume that both somatic and visceral afferent fibers carry impulses which affect a common pool of secondary neurons, and that the principles of summation and inhibition are applicable. Observations like those of Woollard, Carmichael and Roberts (1932) and Livingston (1938) demonstrate that summation of somatic afferent impulses from the shoulder tip area are not essential for reference to that area. On the other hand, the observations of Weiss and Davis (1928) indicate that such a summation of somatic and visceral afferent impulses may be essential for referred pains in other areas but do not necessarily mean that there is a chemical change in the skin subsequent to sympathetic impulses.

SUMMARY

In the cat and dog, these experiments show that nociceptive sensibility produced by stimulation of the central portion of the diaphragmatic peritoneum depends upon afferent conduction in sensory fibers in the phrenic nerve, independent of afferent fibers in the vagus and intercostal nerves and of efferent sympathetic pathways. Observations made on man by Doctor W. K. Livingston are presented to show that pain may be referred on stimulation of the central diaphragmatic peritoneum to a completely anesthetized area of skin in the shoulder-tip region. Viscero-cutaneous reflexes are not essential for nociceptive sensation when the central diaphragmatic peritoneum is stimulated in the experimental animal or in man.

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THE PHASIC RESPONSE TO CORTICAL STIMULATION*

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DURING the further study of extrapyramidal function by the method of multiple stimulation (Mettler, Ades, Lipman, and Culler, 1939) it became apparent that the nature of the 'phasic' response, obtainable in cats on cortical stimulation, required further elucidation. The present study is directed towards this end.

Two types of response may be elicited on faradic stimulation of the cerebral cortex. In the one, a new posture is assumed and held while the electrode is in place; in the other, a pendulum-like movement results. These two responses may be called 'tonic' and 'phasic' respectively. The depth of ether anaesthesia was found to be a significant factor in determining the type of response. When the animals were only lightly anaesthetised phasic movements alone could be elicited. As the anaesthesia was deepened a stage was reached when the phasic character was lost and only tonic responses could be evoked. This observation would suggest that the obtaining of a tonic movement may be dependent upon the removal of a neural mechanism acting normally. If this is so, then our inability to obtain anything but a tonic response in a few cats may be attributed to the poor condition of the animal. Indeed, it has been our experience that haemorrhage from cortical vessels or injury of the superior sagittal sinus is usually present in such a case.

A further factor in determining the character of the response is the strength of the stimulus. Thus, the weakest effective faradic current will produce a tonic movement of small extent, while an excessively strong one will cause the limb to assume the position of full flexion and to exhibit a coarse tremor. Intermediate strengths produce the typical phasic activity. Because of this relation between the stimulus strength and the response, and since the excitation threshold of the cortex fluctuates, an optimum response was always sought by varying the strength of the stimulus. It may be pointed out that although the location of the point stimulated determines the muscles involved in a given movement it does not determine whether the response shall be tonic or phasic. Moreover, even where a unipolar electrode was substituted for the bipolar usually used, see below, the response was still phasic. It is, however, appreciated that even under such circumstances the area stimulated may be relatively gross.

The problem, then, which presents itself in regard to the phasic re-

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sponse, is this—How can a continuous faradic stimulus give rise to a periodic response? Certainly somewhere between the cortex and the primary motor neurons a mechanism is interpolated which serves to direct the train of excitatory impulses alternately to the agonists and the antagonists. The present study offers experimental evidence which indicates (1) that proprioceptive impulses from the limb itself are responsible for the phasic character of the response; (2) that the ventral spinocerebellar tract may be an essential part of the mechanism concerned.

PROCEDURE AND RESULTS

Adult cats were anaesthetised with ether and the cerebral cortex of both hemispheres exposed. The animals were supported in a horizontal position with the legs hanging freely. The motor area was stimulated using an inductorium and a bipolar silver electrode (2 mm. separation). The depth of anaesthesia and the strength of stimulus were adjusted until a continuous application of the stimulating electrode gave rise to a phasic response. Movement of the foreleg, being relatively easy to elicit, was used exclusively for this study. The experimental procedure was varied as follows:

1. *Cutting the dorsal roots of the nerves of the foreleg.* This was done in order to determine if afferent impulses from the foreleg itself were responsible for the phasic activity. In this experiment the spinal cord was exposed and the dura mater opened from the level of segment C3 to Th2. The cortex was stimulated and a phasic response of the contralateral foreleg obtained. The dorsal roots of the nerves of C5, 6, 7, 8, Th1, of this foreleg, were then cut and the cortex stimulated again. The response was now quite different: the leg no longer showed rhythmic movement but instead was drawn up in full flexion and held there without a tremor. Stimulation of the opposite cerebral cortex still elicited a typical phasic movement in the forelimb with intact afferents. If the stimulation of the cortex innervating the operated limb were continued the leg remained steadily flexed for 10 to 15 seconds and then began to relax in steps, cogwheel-fashion, so that after 25 seconds no contraction was discernible in spite of the uninterrupted cortical stimulation. The same treatment of the opposite cortex caused the phasic movement in the normal limb to continue with a constant amplitude for about 10 seconds and then to subside during the next 15 seconds to zero.

2. *Isolation of the forelimb with retention of its blood supply but with all nerves cut except the branch to a single muscle.* In order to identify the source of the afferents responsible for the phasic activity all the muscles and other soft tissues connecting the foreleg to the trunk were severed except the axillary vessels and the brachial plexus. The leg was retained in its normal position by fixing the scapula in a clamp and all the muscles connecting the upper extremity to the trunk were either removed or denervated. The contralateral cortex was then stimulated and a phasic movement of the elbow joint was obtained. At this point all motor and sensory nerves to the limb except the nerve twig to the biceps, were cut and the cortex stimulated

again. In spite of this, a phasic flexion of the elbow due to rhythmic contractions of the biceps was elicited.

In other animals the experiment was modified so as to keep intact the innervation of the triceps instead of the biceps. Again a phasic movement of the passively flexed elbow was obtained—this time by the periodic contraction of the triceps. Since the only source of afferents in the first experiment was the biceps and in the second the triceps, it follows that the impulses responsible for the phasic activity are proprioceptive and arise at least in part within the contracting muscles themselves. An additional observation having a bearing on the nature of the particular afferents involved may be of interest. An experiment was performed in which novocaine was applied to the segments of the cord supplying the forelimb and the response to cortical stimulation tested until paralysis occurred. It was found that at a time when the limb did not react to painful stimuli it still showed phasic activity.

3. *Cutting the dorsal columns.* Having gained some insight into the nature of the afferents it became of interest to try to identify the tracts within the cord essential to the phasic mechanism. To that end with the cervical cord exposed the dorsal columns were fulgurated at the level of segment C4, using a fine wire loop heated to a red heat. The procedure was to make deeper and deeper cuts testing after each one for a change in the response to cortical stimulation. The experiment demonstrated that cutting the dorsal columns, *i.e.*, the fasciculi gracilis and cuneatus, does not abolish the phasic character of the movement; indeed it continued unaltered until paralysis occurred due apparently to encroachment upon the corticospinal tracts. These lesions were checked histologically. (Fig. 1.)

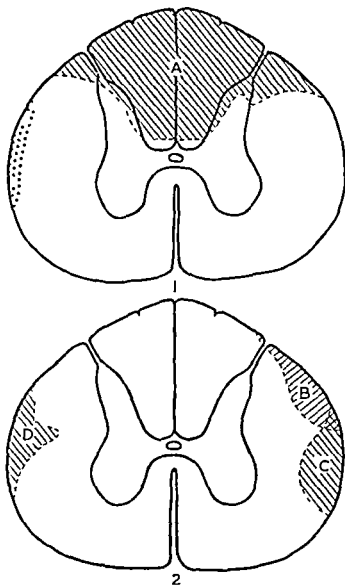
4. *Cutting the spinocerebellar tracts.* The remaining important ascending pathways for proprioceptive impulses within the cord are the tracts to the cerebellum, *i.e.*, the dorsal and ventral spinocerebellar tracts. These were now investigated. The cord was exposed from the level of segments C3 to Th2 and then, after cutting the dorsal columns without abolishing the phasic nature of the response, the wire loop was applied to the cord at the 4th cervical segment over an area on the lateral funiculus corresponding to the extent of the cerebellar tracts. Here a very superficial fulguration served to abolish the phasic responses on the same side. Stimulation of the cortex now produced a tonic flexion closely resembling that resulting from cutting the dorsal roots. As a control the opposite cortex was stimulated and a typical phasic response obtained on the normal side of the cord. Experiments were also performed in which the spinocerebellar tracts were fulgurated without previously cutting the dorsal columns. The same result was obtained, *i.e.*, the phasic response gave place to tonic flexion. It may be pointed out that the character of the maintained flexion following dorsal root section differs from that following either unilateral or bilateral section of the spinocerebellar tracts, since in the former the development and cessation of contraction are more abrupt.

5. *The relative importance of the dorsal and ventral spinocerebellar tracts.*

FIG 1 and 2 Cross sections of the spinal cord at the 4th cervical segment illustrating the anatomical findings Figure 1, area A, shows the most extensive lesion produced in the dorsal funiculus without paralyzing the limb. The lesions in this region unfortunately were made so wide that the deeper ones involved the lateral funiculus and caused paralysis. The few fibres remaining dorsal to the central canal here may have escaped injury but it is unlikely that they could be functional since their blood supply would be cut off (Herren and Alexander 1939). Actually the phasic activity was somewhat enhanced by this lesion.

Figure 2 shows the lesions made in experimental animal No. 32. Lesion B was first made on the right side without changing the phasic response of the forelimb. Cut C was then made and converted the phasic response on the same side into a tonic flexion. A phasic response could still be obtained on the left side by stimulating the opposite cortex. Lesion D was then made on this side and the rhythmic response gave place to a maintained flexion.

From a study of 22 lesions made in the lateral funiculus the location of the ascending fibres responsible for the phasic response is believed to be as indicated by the extent of the dotted area in Fig. 1. This is the position of the ventral spinocerebellar tract (Pass, 1933, Fig. 15). No lesions were made in the ventral funiculus.



A series of small superficial lesions was made beginning at the entrance of the dorsal roots and working laterally and ventrally round the periphery of the cord. The character of the response was tested following each lesion. The response remained unaltered until the region of the ventral spinocerebellar tract was invaded. When the position of that tract was reached tonic flexion alone was obtainable. In a further series of animals single isolated lesions (Fig. 2), were then made in this region, with the same result, *i.e.*, immediate abolition of the ability to react phasically. Thus it was found that the afferent fibres of the mechanism concerned travel up in the region indicated by the dotted area on the left side of Fig. 1 and that they are predominantly uncrossed. Since there is evidence (Pass, 1933) that the dorsal spinocerebellar tract is concerned particularly with the lower limb, this finding serves to contribute the additional information that the ventral spinocerebellar tract is concerned with the forelimb. We do not wish at this time to make any comment concerning the implication which such a finding may have upon the cells of origin of the ventral spinocerebellar tract.

COMMENT

The results of the experiments here described indicate that when a muscle contracts it excites certain intramuscular endings which tend re-

flexly to bring about a cessation of that contraction. This automatic self-inhibition appears to be the chief factor in the production of the phasic activity, although the impulses arising in the stretched antagonistic muscles may also play a part. The afferent nerve fibers concerned, for the forelegs, enter the cord via the dorsal roots of the brachial plexus. Within the central nervous system the pathways, followed by the impulses responsible for the phasic activity, extend beyond the segments innervating the foreleg. This is demonstrated by the finding that a small lesion in the lateral funiculus at the 4th cervical segment (lesion D, Fig. 2), one segment above the origin of the fibres of the brachial plexus, is sufficient to abolish the phasic response. A similarly located lesion at the 2nd thoracic segment, however, even if bilateral has no effect, so it is evident that the mechanism requires at least the higher levels of the cord for its proper functioning. Since the loss of the ability to elicit a phasic response can be induced by very small lesions in the region of the ventral spinocerebellar tract, one is led to think of a possible cerebellar mechanism.

That the loss of phasic activity may result from the general disturbance of cord function, due to the effect of the operation, can be ruled out, since unilateral lesions still permit a normal response on the unoperated side. Moreover, even if a bilateral fulguration of the fasciculi gracilis and cuneatus be carried out (Fig. 1), the ability to respond phasically is usually enhanced and never diminished.

In regard to the possibility that the discharge from the cortex may be periodic during continuous faradic stimulation, it may be pointed out that simply cutting the afferents from the limb which is responding phasically alters the response to one of maintained flexion. This indicates that the train of impulses produced by this stimulation is a continuous one. It serves also to demonstrate that the mechanism responsible for the rhythmic discharge is quite distinct from that involved in the scratch reflex, since cutting the dorsal roots of the nerves to the limb in that case leaves the rhythm of the reflex undisturbed (Sherrington, 1920, p. 251, and confirmed by us in a single experiment performed on a dog).

The question may be raised as to why, in the case where the dorsal roots were cut, the response obtained is always one of maintained flexion and not extension. This is particularly intriguing in view of the fact that if, as shown above, the limb is denervated except for the twig to the triceps then cortical stimulation will cause the triceps to contract. We were unable to perform the experiment of cutting the dorsal roots in such a preparation. In this connection we may quote Sherrington (1920, p. 293), "In the cat, it is in my experience quite infrequent to obtain primary extension of the crossed elbow from the cortex." This also was our finding and its explanation awaits further study.

On the basis of our findings we may now attempt to formulate the mechanism responsible for the phasic response. Peripherally, it consists of afferent neurons whose dendritic endings are within the muscles and whose

central processes pass into the cord via the dorsal roots. These neurons, on being excited by muscle contraction, tend to bring about an inhibition of this contraction. Centrally, the axons of these neurons presumably terminate in the grey matter of the dorsal horns but at any rate the impulses, which they convey, ascend in the cord on the same side in the region of the ventral spinocerebellar tract. No data concerning the course of the fibres at higher levels are available, but, from the evidence pointing to the involvement of the ventral spinocerebellar tract, it would appear that their destination is the cerebellum.

It is of interest to speculate on the significance of the mechanism here described. The fact that it is responsible for the converting of a simple cortically induced flexion response into an alternate periodic flexion and extension suggests that it may be a factor in reciprocal innervation. In that case, of course, the alternating contractions of flexors and extensors would involve muscle fasciculi so that periodicity would not be manifest. Actually, it is quite possible that the functioning of this mechanism, due to its brake-like action, may contribute toward the steadiness and precision of normal movements.

SUMMARY

Two types of response may be elicited in cats on cortical stimulation, depending on the depth of ether anaesthesia, etc. In one a new posture is assumed and held; in the other a pendular movement results. These are designated tonic and phasic responses respectively. The mechanism of the latter type was the subject of this study. This was investigated in the forelimb by determining the effect of (1) cutting the dorsal roots of the brachial plexus; (2) completely denervating the limb except for the twig to a single muscle, e.g., the biceps; (3) making a series of lesions in the dorsal and lateral funiculi of the spinal cord.

The results of these experimental procedures showed that, in so far as the peripheral nervous system is concerned, the phasic response was dependent upon inhibitory proprioceptive impulses set up in the contracting muscles themselves and conveyed to the cord by the dorsal roots. The central part of the mechanism was found to involve an uncrossed ascending path which corresponded in position to the ventral spinocerebellar tract. The complete mechanism responsible for the phasic character of the response would therefore appear to include the cerebellum.

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supply. By removing trachea and ... was done in most cases, the manipulation of the micro-electrode was

For standard recording of the ganglionic potential, chlorided silver wire electrodes with rubber shields were used. The inter-electrode distance was 5 to 6 mm., with the distal electrode on the postganglionic nerve trunk, usually crushed. For stimulating, the same kind of electrodes were applied to the sympathetic (preganglionic) nerve, but with greater interelectrode distance. The nerve was carefully separated from the adherent vagus nerve and cut proximal to the stimulating electrodes. Both pairs of electrodes were usually imbedded in congealing paraffine, a method which was found useful in preventing the preganglionic nerve from drying and the ganglion from moving.

Micro-electrodes were made by drawing glass capillary tubes (1.3 mm. in diameter) to a fine terminal aperture. This was done in a special machine in which the length of taper as well as the aperture could be controlled (see Renshaw *et al.*, 1940). The desired size of the opening was then obtained under the microscope by breaking off the tip of the capillary. The tube was filled with 0.9 per cent NaCl solution by suction. When using small micro-electrodes we have found the NaCl solution more satisfactory and easier to apply than a Ringer-agar solution (cf. Renshaw *et al.*, 1938). Finally a chlorided silver wire was pushed into the capillary tube as close as possible to the terminal aperture and the electrode was mounted on a micro-manipulator. The position of the micro-electrode in relation to the upper surface of the ganglion was controlled in several cases by means of a scale and an ocular reading system. The size of the micro-electrodes used in these experiments varied between 5 and 50 μ . The optimal size for recording the activity in single units on preganglionic stimulation was found to be about 10 μ , the resistance being then about 3 megohms.

The electrical recording system (designed by Mr. A. M. Grass) consisted of a capacity-coupled, push-pull amplifier connected with a cathode-ray oscillograph. On account of the high electrode resistance, 10-megohm grid-leaks were used. The frequency characteristic of the system showed attenuation of the low frequencies, marked below 100 cycles per sec.

The stimulating shock to the nerve was derived from a thyatron set, similar to that used by Renshaw *et al.* (1940). To reduce the stimulus artefact a Wagner ground was used between the stimulating leads. The discharge of the thyatron was controlled by the sweep of the cathode ray, which in its turn was released by a hand-operated key. When repetitive stimulation was used the discharging circuit was controlled by a beat-frequency oscillator. Records were taken alternately with the micro-electrode and the wire electrode on the surface of the ganglion, in each case using the gross wire electrode on the postganglionic nerve trunk as a common lead.

RESULTS

General observations. Two factors largely determine the pictures obtained from the superior cervical ganglion with a micro-electrode, namely, the size and position of the latter. If the micro-electrode is inside the ganglionic sheath and a large number of neurons are responding, an electrode larger than 15 μ can hardly reveal the activity of a single unit, but gives the picture of a ganglionic response split up in several groups. Decreasing the strength of stimulus until background activity of remote neurons subsides may bring out some well-defined spikes. But if the aperture of the micro-electrode is smaller (5 to 10 μ) the single spikes are often bigger and well distinguished. Hence, the clearest pictures are obtained with a submaximal stimulus and a small micro-electrode in a suitable position.

The effect of the position of the micro-electrode is the more pronounced the smaller the electrode. Within the ganglion a change of less than 0.1 mm. suffices to change the pattern. When the micro-electrode is on the surface of the ganglion the picture obtained is largely independent of the electrode

size and resembles closely the record obtained with gross wire leads (cf. Fig. 1A and B).

Large waves recorded with the micro-electrode are sometimes seriously disturbing. In several cases the reason for these waves has been found to be

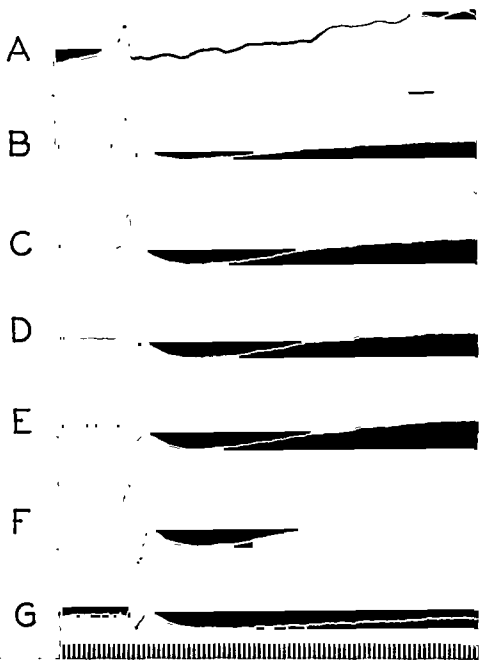


FIG. 1. Standard ganglionic response (B) with gross wire electrodes on surface of ganglion compared with records obtained with micro-electrode (aperture 30μ), with a common reference electrode on the crushed postganglionic nerve trunk. A: micro-electrode on the surface of the ganglion. C to G: micro-electrode pushed stepwise farther into the ganglion. Note similarity between A and B: corresponding increase and decrease of first positive wave and the slow positive after-potential. Maximal preganglionic stimulation. Time in 5 msec. at bottom of figure. In this and all subsequent figures, upward excursion signifies negative potential of the electrode on or in the ganglion.

passive movements of the ganglion due either to respiration or pulse. Further immobilization of the ganglion or choosing another spot for the micro-electrode has often eliminated this disturbance. When the big waves (often

of the order of millivolts) have subsided there still remains a low-voltage slow oscillation of the baseline of unknown origin (cf. Eccles, 1936).

Figure 1 shows some typical records obtained with a large micro-electrode in different positions compared with the standard ganglionic response obtained with gross wire leads (B, hereafter called "standard lead"). In Fig. 1A the micro-electrode is on the surface of the ganglion and the record is strikingly similar to that in Fig. 1B, obtained with standard lead, but as soon as the electrode penetrates the sheath the shape of the response is largely determined by the position of the electrode, as seen in Fig. 1C to G. A large positive wave following the spike potential is often a characteristic of the micro-electrode record. Its beginning and maximum are to a large extent determined by the position of the micro-electrode. It appears in close relation with the preceding spike potential. The slow positive after-potential also seems to be related to this early positive wave. This is indicated in the records by a corresponding increase or decrease in both waves. Their mutual dependence may, however, be obscured when smaller micro-electrodes are used because the frequency characteristic of these electrodes attenuates greatly slow waves such as the positive after-potential. Part of the early positive wave may be due to a ganglionic surface potential led off from the postganglionic lead. Yet the great variability of its duration and latency of the spike maxima indicates that the early positive wave differs in many respects from the main ganglion potential. Furthermore the early positive wave changes its size and latency as the micro-electrode is moved. The change, however, is less pronounced than that of the negative spikes preceding it.

Between the early positive wave and the slow positive after-potential a negative deflection is generally recorded. This may in some cases be of a considerable size and reach the baseline or even cross it before it is succeeded by the slow positive after-potential.

In general the activity revealed by a micro-electrode inside the ganglion is of a considerably greater voltage than the ganglionic response as obtained by one electrode on the surface and the second on the crushed postganglionic nerve trunk.

"Axon-like" spikes. When the aperture of the micro-electrode is made smaller the main ganglionic response becomes progressively more broken up into briefer excursions, and new groups of spikes appear until a state is reached in which the recorded action potentials show an all-or-none character. In the superior cervical ganglion such spikes are best recorded with a 7 to 10 μ electrode. With a maximal preganglionic stimulus spikes can be obtained from almost any part of the ganglion and they may occur after any latency within the range of the standard ganglionic record. Only a few spikes in each record are easily recognized, however, and in most cases only one spike is of considerable height. In Fig. 2 are shown some typical records obtained with maximal preganglionic stimuli. It is obvious from Fig 2B that the diffuse background activity from remote neurons to some extent

obscures the single-spike potentials. However, by moving the micro-electrode it is sometimes possible to record an almost uncomplicated single spike, as in Fig. 2D.

In the experiments in which submaximal stimuli have been used, it was found necessary to explore different parts of the ganglion at different depths with the micro-electrode until the local activity of a single unit was recorded.



FIG. 2. Maximal preganglionic stimulation. A: ganglionic response with standard lead (gross wire). B, C and D obtained with a micro-electrode of 10μ aperture in different positions within the ganglion. Amplification the same in all records ($200\mu V$, shown by vertical line in A). Time: small squares in abscissae equal to 1.4 msec. Time of stimulus shown by artefact at the left of each record.

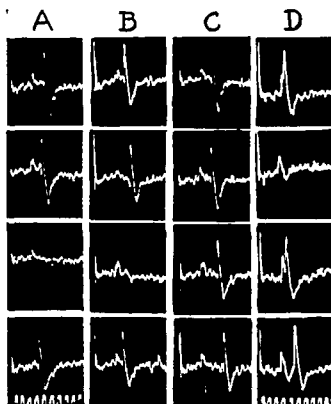


FIG. 3. "Axon-like" spikes recorded with unaltered position of the micro-electrode (aperture 7μ) but with different strengths of preganglionic stimuli, increasing from A to D, but still submaximal. The records in each vertical column were taken in one sequence without change in the strength of stimulus at an approximate frequency of 1 in 10 seconds. Note the all-or-none character of the spikes as well as the great variability in latency. Stimulus artefact appears at the left in nearly all records. Time in 2 msec.

Figure 3 shows a sample of spike potentials recorded with a 7μ micro-electrode and submaximal stimuli.

The all-or-none character of the recorded spikes is emphasized by the fact that their size or shape is independent of the strength of the stimulus and by their complete absence in some records taken in one sequence (cf. Fig. 3A, B, C, D). This sudden failure is also seen during a repetitive stimulation at maximal strength sufficient to depress greatly the ganglionic response in a few minutes. In these cases the spikes, superimposed on the

ganglionic response, suddenly drop out after a certain time of stimulation.

The duration of the spike potentials as measured at the level of the baseline varies between 1 and 2 msec. and is constant for the spike response to successive stimuli with the micro-electrode in an unaltered position. The size of these potentials may approach $500\mu\text{V}$. and is often greater than the corresponding ganglionic potential recorded with surface leads (cf. Fig. 2A and D).

The latencies of the spike potentials, as measured from the stimulus artefact (4 to 5 cm. preganglionic conduction), have shown a wide range covering the usual duration of the ganglionic potential. Spikes with longer latencies are usually found at the cephalad end of the ganglion, but latencies up to 20 msec. have also been recorded from the caudal part. In most of the cases the latencies are found to correspond to the maximum height of the ganglionic response with standard leads. Yet significant variations in the latency of the individual spike potential have also been observed. Thus for instance the latency of the potential seen in Fig. 3, varied between 7 and 12 msec. This variation was studied especially with submaximal stimuli and was found to be largely independent of the frequency of the stimuli. Thus the variation occurred when the preganglionic nerve trunk was stimulated at rates varying from 1 per min. up to 40 per sec. That this variation was not due to the excitation of different preganglionic fibers was indicated by the fact that a variation of 7 msec. of the latency was observed when maximal stimuli were used. Yet in spite of the fact that the stimuli were maximal, this spike potential was often absent. The cause of this failure could not be determined. It occurred at low frequencies (1 per min.) as well as at high. As the stimulus frequency increased, the irregular failure of individual responses became more frequent. The relation between the resulting total frequency of response and that of stimulation is shown in Table 1. Yet even when this correlation was at a minimum, response followed stimulus with latencies showing only the above-mentioned variations. When the rate of stimuli was 40 per sec., the spike potential disappeared from the record in about 30 sec. and at a time when most of the ganglionic potential had disappeared.

With respect to the shape of the spike potentials there is usually an indication of a break at its descending phase (cf. Fig. 3). After this break the drop of the potential is less rapid and this late phase of the spike is followed by the first positive wave, which, though slower than the negative spike, is much more rapid than the positive after-potential. The magnitude of this early positive wave, as well as its duration, shows great variability with respect to spikes recorded from different positions and also to successive spikes recorded with unaltered electrode positions. Its size may be as much as half of the preceding negative spike and its average duration 2 to 6 msec. A relationship between this early positive wave and the slow ganglionic after-potential has been indicated above.

Besides the spike potentials the record shows a low-voltage spike-like

activity which is at times indistinguishable from the noise of the amplifier. The magnitude of these spikes is usually not more than about $50\mu\text{V}$. The latency to the earliest group of these low-voltage spikes is about 5 msec.; they constitute the most persistent part of the micro-electrode record during repetitive stimulation. A careful study of the records reveals in several cases a fast regular rhythm (about 1000 cycles per sec.) immediately preceding the main spike potential and at the same time or separately a slowly rising potential.

In the absence of electrical stimuli, a slowly subsiding injury discharge has often been observed when the micro-electrode is pushed farther into the ganglion (Fig. 4). This discharge is especially clear when small micro-electrodes are used, and may take the form of a single-spike potential. These spikes are, however, often followed by very large positive "after-potentials." The injury discharge may even lose the negative spike while the positive wave still continues to appear (see Fig. 4).

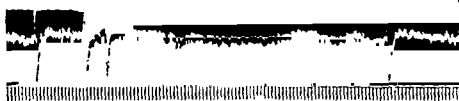


FIG. 4. Injury discharge (no electrical stimulation) obtained with a micro-electrode of 10μ aperture. Note fast "axon-like" spikes followed by large positive waves and the appearance of positive wave without a preceding negative spike. Time in 2.5 msec.

A few experiments were carried out on rabbits and essentially the same kind of records were obtained. Several attempts have also been made to reveal a single-spike activity with micro-electrodes inside the pre- or postganglionic nerve trunks. While spikes of a low voltage hardly exceeding $50\mu\text{V}$. were recorded together with the integrated potential, none of the spikes were comparable to the large spikes obtained with micro-electrodes in the ganglion.

DISCUSSION

With a similar micro-electrode technique Renshaw *et al.* (1938, 1940) have recorded fast "axon-like" spikes from the cell-layer of the hippocampus region. These potentials were interpreted as due to the activity of the cell bodies but not necessarily confined to a single unit. A similar point of view has also been suggested with respect to the spike potentials recorded from the superior cervical ganglion (Therman and Forbes, 1939). The present study emphasizes the all-or-none character of the spikes in the ganglion. With respect to their possible point of origin all the available data strongly support the view that the "axon-like" spike potential represents the discharge of a single cell. It is evident that the spikes are postsynaptic phenomena, both because of their disappearance during prolonged repetitive

stimulation (cf. Orías, 1932; Rosenblueth and Simeone, 1938) and the great variability of the latency. That they cannot be due to the action potentials of presynaptic myelinated nerve fibers is indicated by the latency, which in some cases may exceed 20 msec., while responses conducted along those fibers should appear among the group of M_1 spikes (Bishop and Heinbecker, 1932) with a latency of less than 10 msec. Because it has not been possible to record similar spike potentials from either the preganglionic or postganglionic nerve trunks it is difficult to attribute those spikes to the activity of unmyelinated nerve fibers. Furthermore, the spike-duration is shorter than generally assumed to be the case in C fibers (Bishop, 1934; Gasser, 1937, 1938). The suggestion that these "axon-like" spikes actually are due to the electrical activity of a single cell is strongly supported by their all-or-none character and brief duration (1.5 to 2 msec.). Furthermore, they are best recorded with small micro-electrodes with an aperture of 7 to 10μ and are sharply localized. Thus the ganglion cell remains as the most probable source of these spike potentials; in fact we have been unable to find any other explanation which would account for our observations. The size of the most common type of cell in the superior cervical ganglion ranges between 15 and 30μ (de Castro, 1932). It is likely that a small micro-electrode (e.g., 10μ in diameter) close to the cell surface is capable of recording a localized potential gradient with respect to a remote point on the postganglionic nerve trunk. This is emphasized in most of the records in which the spike potentials actually are bigger than the integrated ganglionic potential as obtained with surface electrodes. The large injury potentials may also be recalled in this connection as indicating that the position of the micro-electrode is close to their point of origin.

The great individual variability of the latencies of some single-spike potentials is significant. Although the frequency of the stimulus was not more than 1 in 10 seconds, in one case (submaximal stimuli) the latency varied between 7 and 12 msec., in another (maximal stimuli) between 12 and 19 msec. Effects of preceding excitation lasting even a minute or more have been demonstrated by Bronk and his associates (Bronk, 1939) in the stellate ganglion. Also the multiple innervation of ganglion cells may be recalled in this connection (cf. Eccles, 1935b), but the ultimate cause of these changes in latencies remains obscure.

The experiments with repetitive stimulation have clearly emphasized the fact that the postsynaptic neurons may discharge at rates which do not correspond to the frequency of the preganglionic stimulation (cf. Bronk, 1939). Although the discharging frequency never was higher than about 4 per sec. and actually became slower at higher stimulating frequencies (cf. Table 1), a complete block was obtained on continued stimulation (cf. Bronk and Pumphrey, 1935; Rosenblueth and Simeone, 1938).

With respect to the sequence of potential changes of active single units recorded with micro-electrodes, the method does not yet permit any conclusive comparisons with known features of after-potentials in peripheral

nerves. It can merely be pointed out that the break in the descending phase of the single-spike potential indicates the presence of a negative after-potential electrically comparable with the negative after-potential of nerve. This negative after-potential passes over into the early positive wave, which is separated by another negative deflection from the slow positive ganglionic after-potential. These two positive waves have been shown to be related in size, but are always separated by a negative wave. The potential sequence from the first positive wave through negativity and ending in the slow positive after-potential resembles the two positive after-potentials in nerve described by Gasser (1935, 1938); but it should be noted that whereas the

Table 1*

Frequency of stimulus (Sec.)	Frequency of response	Duration of record (Sec.)
8 max.	0 4	12
18 max.	1 9	7
18 submax.	1 3	7
18 max.	1 6	9
30 max.	3 8	7
40 submax.	2 4	8
40 max.	2 4	10

* All measurements in seconds.

early positive wave of the ganglionic potential lasts about 5 msec., the first positive after-potential described by Gasser may last as much as 50 msec. There is the possibility that diphasic effects may play a part in determining the observed form and duration of the waves. We have not the data with which to analyze this possibility.

The persistence of an early group of low-voltage spike potentials during repetitive stimulation suggests that the micro-electrode to some extent is able to record the electrical activity of presynaptic fibers. Delayed small spikes may again partly be due to activity in postsynaptic fibers.

SUMMARY

The electrical activity of the superior cervical ganglion of cats and rabbits has been studied with micro-electrodes. The recording system consisted of a capacity-coupled, push-pull amplifier and a cathode-ray oscillograph.

"Axon-like" spikes are obtained during preganglionic stimulation or as an injury discharge with micro-electrodes of 7 to 10 μ apertures. The spikes are postsynaptic phenomena and show an all-or-none character and a duration of 1.5 to 2 msec. They are found dispersed over the time range corresponding to the standard ganglionic record.

The brief duration, sharp localization and all-or-none character of the spikes recorded with the smallest micro-electrodes strongly suggest that they are derived from single cells.

Study of the single-spike potential during successive stimuli and with

an unaltered position of the micro-electrode has shown variations of latency as great as 7 msec. In some cases the frequency of response is different from and largely independent of the frequency of the preganglionic stimuli.

The "axon-like" spike is followed by one negative and two positive after-potentials, the last being identical with the slow positive after-potential of the ganglionic record.

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EFFECT OF CORTICAL LESIONS ON AFFECTIVE PUPILLARY REACTIONS*†

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THE WORK of Déjerine (1906), Roussy (1907) and Head (1912, 1920) has established the importance of the thalamic level for pain sensibility. That pain may enter into awareness at the thalamic level was first stressed by Déjerine, and Roussy described the "syndrome thalamique" in which thalamic lesions cause contralateral hemianesthesia, hemiataxia, choreoathetoid movements and intense but poorly localized pain. Head utilized his clinical observations to extend to sensory activity the concept of "levels of function" which Hughlings Jackson had applied principally to the motor apparatus. Head maintained that stationary lesions involving the somatic sensory areas of the human cortex did not raise the threshold for pain perception, while such functions as light touch, two point discrimination, stereognosis and the discrimination of fine temperature differences may be markedly diminished or abolished. These studies have since been extended by Penfield (1937) who agrees that pain has little or no cortical representation. The concept of "thalamic release" was further advanced by Head to those cases in which cortical lesions caused a heightened response to pleasurable as well as to painful stimuli. Evidence that central pain of organic as well as of psychogenic origin may be caused by thalamic hyperactivity has been presented by Foerster (1927) and also by Storrington (1938).

By the method of local strychninization Dusser de Barenne (1931) demonstrated a gross localization of somatic sensibility in the thalamus of the cat. In later studies (1938) on the monkey he indicated that sensory activity depends upon a reciprocal thalamocortical system. Complex pathways capable of inhibiting the thalamus by way of the cortex and striatum were delimited. These previous studies suggested the need for a simpler experiment capable of separating the function of the thalamus from that of the sensory cortex, and which could perhaps serve as a "thalamic indicator" in the intact animal. To this end the following method was devised.

METHOD

Adult cats of 2-3 kg. were used. The brain was exposed by a subtemporal approach, and the sensory area of the contralateral hind leg, as delimited by Dusser de Barenne (1916) and by Bard and Brooks (1934) was removed, either by a lateral incision into the brain substance, or by electrocoagulation. This sensory area comprises the rostral half of the longitudinal gyrus (lateral gyrus of Winkler and Potter). Part of the laterally adjacent supra-Sylvian gyrus, and that part of the sigmoid gyrus lying back of the excitable hind leg motor area was also removed. In 2 animals the motor area was included, and in 2 others more extensive cortical lesions were produced. In all animals the legs deprived of

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cortical representation showed a permanent loss of the dorsal placing reaction of Bard with preservation of the reactions on the normal side.

On each of these animals the following experiments were begun 3 weeks after the operation. The animals were placed in a box with a head holder which kept the head fixed, but allowed free observation of the eyes. After a variable period of training, lasting from 3 to 25 days, the animals would remain quiet throughout the experiment. Thus, no anesthesia was necessary. Unipolar electrodes were attached to the legs, making contact at the middle toe pad. As a stimulus an alternating current one sec. in duration upon which a constant "break" of 8 to 10 times per sec. was superimposed, passed through an inductorium with the hammer out of circuit. All sources of conditioning to the stimulus were



FIG. 1. Photographs of cats in experimental set-up, A. Before stimulation, and B. After stimulation.

avoided. The alternate thresholds required to elicit pain reaction from stimulation of both normal and cortically ablated legs were then recorded at one minute intervals. The pain reaction chosen as the indicator was the reflex dilation of the pupil which is mediated solely by central inhibition of the Edinger-Westphal nucleus.* As a control for the present experiment 2 cats were prepared with unilateral vago-sympathectomy. Both eyes responded with dilation at the same threshold, proving that the threshold was determined by a central inhibition, which evidently is capable of reflecting changes in the higher levels of the neural axis.

The animals with cortical lesions were placed in the box, and the head illuminated by a strong, constant light. The eyes were observed through a low-power microscope, fitted with a graduated scale, on which one division equalled 1 mm. of pupillary diameter. A dilation of one division was considered sub-threshold, and a dilation of 2 divisions as the threshold reaction (Fig. 1). This reaction occurs within the second of stimulation, and is reversible in from 1 to 2 seconds. The pupillary reaction is the constant and unfailing precursor of more violent pain reactions, such as moving or crying. A shortening of the coil distance, 0.5 cm. from the point which gave the threshold pupillary reaction, gave

* That this reaction is exclusively an inhibition has been stated by Bechterew (1883), Braunstein (1893) and Bain, Irving and McSwiney (1934). Ury and Gellhorn (1939) have extended these observations.

these stronger, more generalized reactions. Both eyes react simultaneously and to the same degree, irrespective of the leg stimulated. Under these experimental conditions the dilation of the pupil in response to an afferent stimulus is a constant, sensitive indicator of a central process associated with pain. It is caused by a central inhibition, and is uncomplicated by any variable factors in the periphery.

The preceding method was devised to differentiate the role of the cortical somatic sensory areas from that of subcortical sensory levels, especially the thalamic. However, since Karplus and Kreidl (1910) obtained pupillary dilation from stimulation of the frontal cortex of the cat and from area 8 of the monkey, an additional method was used to study this cortically initiated reaction. Cats were prepared similar to those used by Ury and Gellhorn (1939) in which one pupil was deprived of its oculomotor innervation and constricted with eserine, while the other was sympathetomized. Five days post-operatively the the calvarium was removed under local novocaine infiltration and the cortex stimulated by bipolar electrodes, 1 mm apart. The cortical areas giving threshold pupillary reactions by either oculomotor inhibition or sympathetic discharge were then plotted.

RESULTS

Results of individual experiments on animals with cortical lesions are best expressed by the submitted graphs (Fig. 5 and 6). The following table summarizes the results.

Table 1

Animal No	1	2	3	Total
4	13	1	2	16
5	10	3	1	14
6	10	0	1	11
7	9	1	1	11
10	0	8	0	8
11	7	0	1	8
8	13	2	3	18
14	10	0	0	10
12	3	11	0	14
17	4	8	1	13
	79	34	10	123

Column 1 Number of experiments showing no differences in reaction on either side

Column 2 Number of experiments showing ablated side more sensitive

Column 3 Number of experiments showing normal side more sensitive

Animals with sensory ablation 4, 5, 6, 7, 10, 11

Animals with sensorimotor ablation 8, 14

Animals with massive lesion 12, 17

Sixty-one experiments were performed on 6 animals which had ablation of the sensory cortex. Of these, 13 experiments showed the ablated side to be more sensitive by 0.5 cm on the threshold determination. Of these latter, 8 experiments occurred in Cat 10 which showed a small but constant increased sensitivity on the ablated side. At autopsy nothing unusual was found to account for this fact. Six experiments showed the normal side to be slightly more sensitive, 49 experiments showed no difference.

Twenty-eight experiments were performed on 2 animals with sensorimotor lesions. Of these, 4 showed the normal side more sensitive, and in 2 the ablated side responded at the lower threshold, while 22 showed no difference.

Twenty-six experiments were carried out on the 2 animals which survived extensive cortical lesions. Of these, 19 showed a marked lowering of the threshold on the affected side, and one isolated experiment showed the normal side more responsive.

All the lesions were verified by stimulation of the post-cruciate motor area either on the normal or affected side. The brain of one animal of each group was sectioned and every tenth section stained alternately with Nissl and Weil's myelin sheath stain. The sections were cut in the plane indicated by the monograph of Winkler and Potter (1914), whose nomenclature and topographical divisions are utilized in this paper.

HISTOLOGICAL STUDIES

CAT 4 was selected as typical of the animals with sensory lesions. Nissl section 180 at the level of Pl. 2 of Winkler and Potter shows the motor area bilaterally intact. Cortical destruction included the longitudinal gyrus with extension to the adjoining posterior portion of the sigmoid gyrus. Medially, the lesion reaches the sulcus splenius. This confirms the destruction of the sensory area of the hind leg as shown by permanent loss of the placing reaction. The subcortical structures are intact, and there is a slight loss of cells in the nucleus ventralis.

CAT 8, one of the animals with lesion of the sensorimotor cortex is shown in Fig. 2. The focal point of the electro-coagulation is in the rostral half of the longitudinal gyrus



FIG. 2. Photograph of brain of Cat 8, with sensorimotor lesion.
C. S., Cruciate Sulcus.

with the destruction spreading to the cruciate sulcus, which is obliterated on the left. Nissl section 250, between Pl. 2 and 3 of Winkler and Potter shows destruction of the cruciate sulcus on the left with the region beneath occupied by a dilation of the lateral ventricle. There is gross destruction of the cell layers in the cortical areas 5 and 7 in which lie the sensory areas of the hind leg, and a destruction of the nerve fibers in the centrum ovale leading to this area. This confirms the physiological evidence delimiting the lesion, which showed a permanent loss of the dorsal placing reaction on both right legs and an extensor rigidity lasting 3 weeks on the right hind leg. All subcortical structures are intact.

CATS 12 and 17 are animals with massive cortical lesions. On Cat 12 an extensive unilateral cortical electrocoagulation posterior to the motor area was carried out by one of us (E.O.) on August 11, 1937. Upon recovery the animal showed a permanent loss of the dorsal placing reactions of Bard on both left legs, while the right responded normally. This animal showed marked extremes of

behavior, either being unusually quiet and docile or extremely excitable. At no time did it show pupillary inequality, and all through the experiments both eyes reacted at the same threshold and to the same degree. This bilateral reaction was evoked at a lower threshold when the hind leg contralateral to the lesion was stimulated. Usually the eye homolateral to the leg stimulated was used as the indicator, but in several experiments a single eye was used throughout without altering the result. The destruction of pupillo-constrictor neurons in the right occipital cortex does not account for the differential obtained in afferent stimulation, but made the entire pupillo-constrictor apparatus more sensitive to inhibition.

The "release" phenomena observed in this animal showed great variability, and in 3 experiments this was not obtained. For a time this "release" seemed to decrease but a later experiment, on May 20, 1938, showed this effect to have regained its initial magnitude.

In these experiments an important factor which determines the threshold is the resistance of the skin to the passage of the stimulating current. Sweating decreases this resistance. To evaluate this peripheral factor, readings were taken of the skin resistance of the toe pads by C. W. Darrow. This showed the resistance of the normally represented leg to be 7,000 ω , while the figure for the cortically ablated leg was 11,000 ω . Thus, any difference in peripheral permeability would increase, and not lower the threshold of the leg with cortical lesion. This result agrees with the work of Bucy (1935), who found that cortical lesions first increase, but later decrease the sweating on somatic areas which they influence.

CAT 12 *Summary of microscopic study.* The animal was anesthetized and the brain exposed. In attempting to remove the adherent leptomeninges, softened brain tissue exuded and an artificial opening was made into the lateral ventricle. After injection with 10 per cent formalin, the brain was removed and routinely sectioned, every tenth section being stained alternately with Nissl's method and Weil's myelin sheath stain.

Extent of the lesion. Grossly, the destruction of the cortical tissue includes the middle and posterior part of the parietal lobe, the occipital and temporal lobes. Microscopic study, however, shows that the lesion begins just posterior to the cruciate sulcus. Section 220, which lies midway between Pl. 2 and 3 of Winkler and Potter, here borders the lesion rostrally. Nissl section 210 shows area prepirietalis 5, which contains agranular cortex, to be normal, while posteriorly area prepirietalis 7, which is the rostral border of the sensory area, is destroyed. Medially the lesion ends at the sulcus splenius, while laterally the gyrus lateralis (longitudinal gyrus of Bard and Brooks) and the gyrus supra Sylvius are destroyed. The caudate nucleus is distorted, being pulled out in a vertical and lateral direction, and at its most dorsal point just touches the lesion, which invades the corona radiata. Microscopic study of the caudate nucleus on the affected side shows marked vacuolization of the ganglion cells, gliosis and neuronophagia due to direct influence of the lesion. The left (normal) caudate shows only slight gliosis. On the normal side the section passes through cortical areas 1, 5 and 7. These areas represent the sensory area of the normal hind leg, and show slight gliosis but no marked abnormality. This confirms the physiological integrity of this area as shown by the placing reaction.

Figure 3 is a reproduction of section 460 stained with Weil's myelin sheath stain. This and contiguous Nissl sections delimit the following pathology. Medially the cortical lesion includes the gyrus supra splenius and sulcus splenius. Laterally the lesion extends to the gyrus Sylvius posterior, including the gyrus lateralis and gyrus Sylvius. There is destruction of the corona radiata and some invasion of the internal capsule.

The form system is bilaterally intact with no invasion of any ventral lying structures, and the periventricular system comprising the nuclei ependymalis, nucleus reuniens and the medial nuclei are bilaterally normal. The right thalamus is slightly smaller than the left, with the following changes in the right thalamus. Nucleus anterior a is one half the size of the control nucleus, its capsule of myelinated fibers is decreased, and there is a marked loss of ganglion cells and gliosis. There is a loss of cells in the ventral portion of nucleus lateralis a, and diffusely in nucleus ventralis b. The lateral geniculate body on the right shows marked shrinkage, a loss of surrounding myelin sheaths, and almost complete loss of ganglion cells. At the caudal portion of the occipital lobe a section corresponding to Pl. 19 of Winkler and Potter shows the cortical lesion to extend from the sulcus supra splenius medialis to the sulcus supra Sylvius posterior. The entire cortex of the left (normal) side shows only a slight gliosis, confirming its physiological integrity shown by the placing reaction.

CAT 17 is the second animal which survived massive cortical ablation. Here the occipital cortex was spared. The operation was done October 18, 1938, and in 5 experiments performed during the succeeding month, 4 showed no difference in threshold between the two sides, and one experiment showed the normal side more responsive. In the 8 succeeding experiments done since November 1938, all showed the ablated side to be more sensitive by a difference of 0.5 to 1.0 cm. on the threshold determination. Rough subjective tests show that in this region of the inductorium a decrease of 1.0 cm. results in a doubling of the stimulus delivered.

area and sensory area of the hind leg. Cortex of the control leg uninvolved (m) Massive
is De
Cortex
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cortical lesion: Destruction of the cortex from the sigmoid gyrus to the occipital cortex on the right. Slight gliosis of the normal cortex. Subcortical involvement on the right as follows: (a) Marked pathologic changes in the caudate nucleus. (b) Marked loss of cells in the anterior nucleus of the thalamus. (c) Destruction of the geniculo-striate system.

To what extent each of these factors is responsible for the experimental result cannot be decided from the data given by one animal.



FIG. 3. Photograph of Cat 12, Section 460, level between Plates 12 and 13, Winkler and Potter.

Five cats were prepared as previously described to determine the effects of cortical stimulation. The results of these experiments will be described more fully later, but their essentials may be reported as follows: (i) Three cortical fields were found which gave the inhibitory pupillary reaction on the sympathetomized eye. These fields correspond in a remarkable way to the regions from which Tower (1936) obtained inhibition of extrapyramidal movement in the cat. Her Fig. 2 also represents our fields a and b. (a) A frontal field lying anterior to the cruciate sulcus in the gyrus proreus. This is the region from which Karplus and Kreidl (1910) obtained their pupillary reaction. This field gave the inhibitory reaction at 9.0 cm. (Harvard inductorium) and bilateral nictitating membrane retraction at 6.0 cm., without any observable sympathetic pupillary response. (b) A field lying in the anterior and posterior sylvian gyrus. This field gave only the inhibitory pupillary response at 7.5 cm. No nictitating membrane discharge was obtained with increasing stimulus. (c) An occipital field, weaker and less extensive than the preceding, which gave only inhibitory responses at 7.0 cm. (ii) No pupillary or nictitating response was obtained from the somato-sensory area of the hind leg, as previously outlined.

DISCUSSION

The preceding experiments indicate the following: (i) The threshold of pupillary response to pain stimuli is not raised by ablation of the sensory or sensorimotor cortex in the cat. (ii) In 2 animals with massive cortical lesions the threshold to pain was lowered. (iii) The inhibitory pupillary reaction may be obtained from cortical areas not related to the somato-sensory system.

At this point the question of a bilateral sensory cortical representation must be discussed briefly. Marshall, Woolsey and Bard (1937) have shown by action currents that the tactile sensibility of the entire body, excluding the face, is represented only in the contralateral cortex. In general, sensory points lie posterior and lateral to the motor points. Bilateral effects are due to diffuse activation of the thalamus either by extreme artificial stimuli or such powerful pharmacologic agents as strychnine. Dusser de Barenne (1935) attributes the bilateral effects he obtained by strychninization of the cortex as due to activation of thalamic nuclei. In the monkey, the limit of the sensory representation on the medial surface is the first sulcus buried in the medial fissure. This is the sulcus splenius in the cat. In the cat, less of the median surface is involved in the sensory radiation, which only represents the area of the perineum and tail, while the flexor surface of the hind leg lies above and lateral to this perineal area.

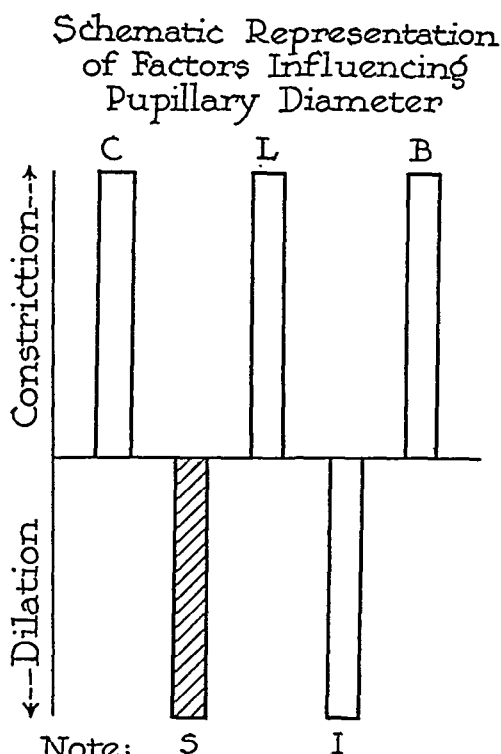
The experiments here reported show that the presence of the sensory cortex on the control side does not add any positive component to the indicator reaction in response to nociceptive stimuli. Therefore this threshold must be subcortically determined. Before defining these determining structures by means of the indicator reaction, a general scheme for interpreting pupillary reactions is presented.

Reactions occurring at the pupil reflect a summation of central events made evident through a peripheral mechanism. These central and peripheral mechanisms may be considered separately if the reacting structures are divided into the following 3 regional groups: (A) Mechanism of the peripheral innervation, (B) Nuclear mechanisms, (C) Supranuclear mechanisms—central pathways.

A. Peripheral innervation. The course of the sympathetic pre-ganglionic fibers to the pupil has been reexamined by Bishop and Heinbecker (1932). Sectioning of this sympathetic pathway anywhere in its peripheral course, and also at any point in its descending bulbar and spinal pathways from the hypothalamus results in a Horner's syndrome whose restitution shows a variable course depending upon the site of the lesion.

The autonomic part of the third nerve is the paired ventromedial nucleus of Edinger-Westphal. Its small-celled anterolateral portion gives off fibers which innervate the sphincter of the pupil. Recent studies by Crouch (1936) show that each iris is innervated by both the homolateral and contralateral portions of the nucleus. Each iris is, therefore, under the control of both crossed and uncrossed fibers. Conversely, each side of the

nucleus influences both pupils. This clarifies the mechanism by which both pupils react as a unit to constrictor impulses in the consensual light reflex and to central inhibition.



The blank columns represent factors summing at the Edinger-Westphal Nuclei. The shaded column represents the effect of the sympathetic fibers

FIG. 4.

following diagram (Fig. 4). *Column S* represents the sympathetic dilator tonus which may be considered invariant. Its effect is peripheral, while the 4 remaining elements enter into events occurring at the Edinger-Westphal nucleus. Their significance is as follows:

Column I represents central inhibition which acts in the dilator direction and is the measure of sensory, affective activity. *Column C* represents the cortical component of pupillary constriction, obtainable in cats upon stimulation of the visual cortex. *Column L* represents the constrictor tonus due to the light reflex, mediated by the pretectal area.

Column B represents a constrictor tonus which differs from *L* in that it is independent of the light reflex. This independent rhythmic discharge represents the "basal tonus" of the Edinger-Westphal nucleus and is seen

None of the variability of the pupil to light or afferent stimuli is lost when the sympathetic component is interrupted, but the magnitude of the reaction is diminished. That normally this difference is due to the tonic action of the sympathetic and not to its reflex excitation has been shown by Ury and Gellhorn (1939). This concept is valid also for the variable dilation in such a preparation upon decreasing the impinging light. Von Brücke (1931) and later Bender (1938) found no reaction to light upon section of the oculomotor nerve. Gullberg, Olmsted and Wagman's (1938) assumption that in dark adaptation the sympathetic tonus increases is further disputed by the clinical fact that in complete internal ophthalmoplegia the pupil is fixed. The variability of the pupil is therefore due to variation in oculomotor tonus, which is in itself a summation of various nuclear events.

B. Nuclear mechanisms. In relation to afferent stimuli all the factors which are summated in pupillary reactions may be represented in the

when complex superimposed pathways projecting excitation and inhibition upon this nucleus are eliminated. These pathways are evidently suppressed in sleep, coma, or deep narcosis, which are characterized by a pupillary constriction maintained in the absence of light. The "paralytic dilation" signifies the cessation of this spontaneous neural discharge and usually the death of the animal. Similar automatic, independent rhythms have been observed in other neurons and neural aggregates. Adrian (1932) led off rhythmic action potentials from the isolated brain stem of the goldfish in regions of the vagal nuclei. Libet and Gerard (1939) demonstrated spontaneous action potentials from isolated olfactory lobes of the frog, even when synaptic junctions were abolished by nicotine. That larger functional aggregates may act similarly is suggested by the Berger rhythm obtainable from the occipital cortex in absence of visual activity, and also from other cortical areas.

C. Inhibitory supranuclear pathways. Central inhibitory processes are usually projections from higher to lower levels, and therefore pathways inhibiting the Edinger-Westphal nucleus should traverse structures lying above the level of the third nerve. Since direct stereotactic stimulation of the hypothalamus causes this reaction (Ranson (1938) and collaborators) this structure may be the immediate effector organ. In preceding experiments with cortical lesions a point to point correspondence with afferent stimuli has been demonstrated. Since this reaction is extinguished by appropriate narcosis (Ury and Gellhorn, 1939a), and is further a constant expression of affective states, these afferents must reach a sensory level which contributes to awareness. The results of Bremer and collaborators (1936), especially E. Class, show that direct stimulation of the thalamus, cortico-thalamic tracts and other diencephalic structures yield this reaction.

The experiments reported on Cats 12 and 17 indicate overactivity of a sensory level to nociceptive stimuli. That this property is essentially thalamic has been stressed by Walker (1938), Head (1920) and Foerster (1927). Should this reaction prove to be an expression of a thalamo-hypothalamic mechanism, various anatomical pathways are at its disposal. LeGros Clark (1932) and Walker (1938) indicate periventricular fibers. Greving (1928) and Huber and Crosby (1929) stress connections by way of the striatum and subthalamus. It is these which Penfield (1929) considers responsible for the autonomic symptomatology in his case of a tumor in the anterior thalamus.

Although the activity of the little known tectal sensory level cannot be excluded as contributing to this reaction, the evidence favors the interpretation that diencephalic mechanisms are principally involved. A modification of the experimental method described here, which would determine synchronous points between the indicator reaction and the electrical activity of a given neural unit, might yield a new method for determining diencephalic relations.

The results obtained here on the somatic sensory system of the cat are

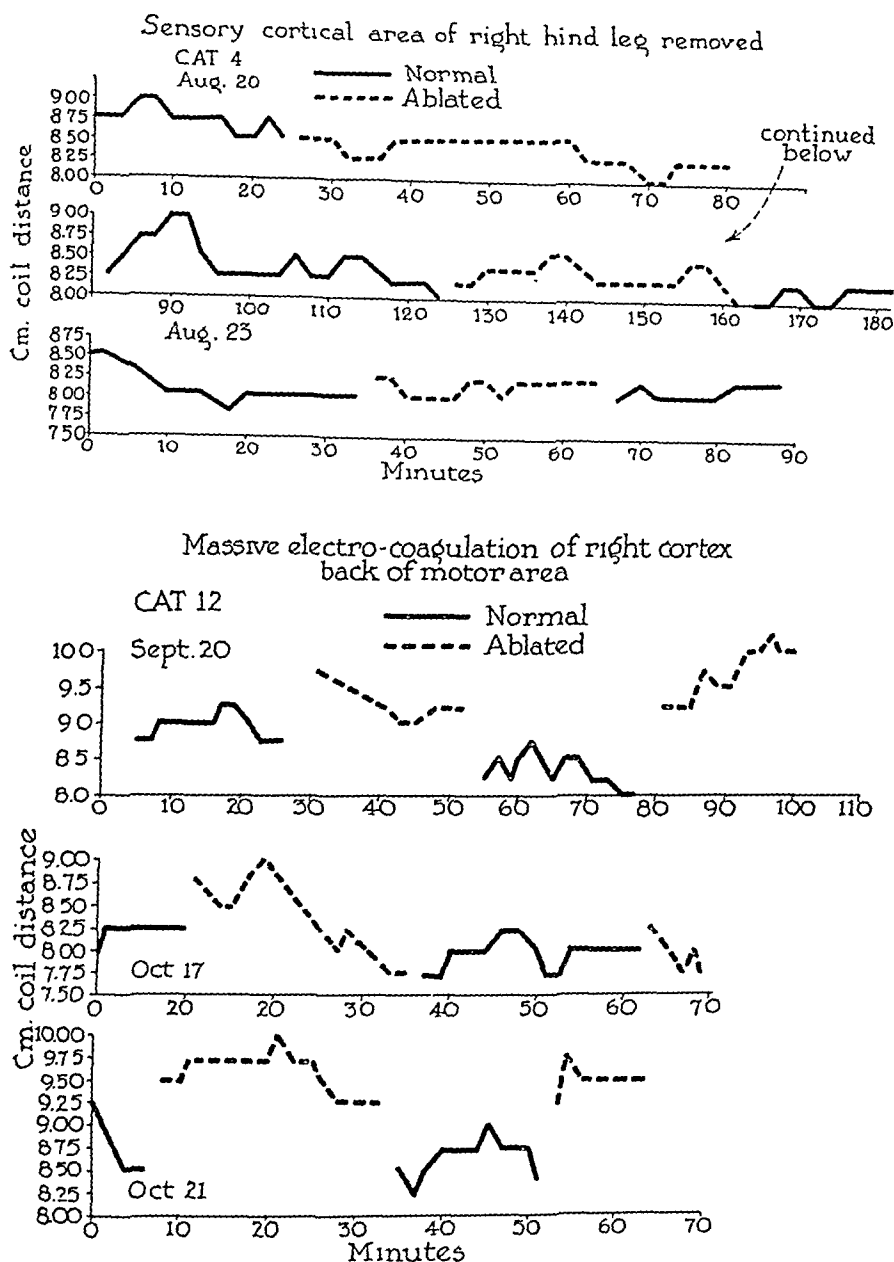


FIG. 5 and 6. The graphs show the coil distance necessary to obtain the threshold reaction. The ordinate is coil distance in cm., the abscissa is time in minutes.

in agreement with other forms of sensory intensity discrimination, especially those relating to the visual system. Lashley (1937) has shown by extended studies on the rat that destruction of the striate area permanently abolishes detail vision, leaving unimpaired the ability to discriminate light intensity,

which is determined by subcortical visual structures. Intensity discrimination is here a subcortical function, while spatial localization and sequential relationships are contributed by the cortex. In man brightness discrimination has undergone corticalization, but it is significant that pain discrimination in man as well as in animals is still effected at the highest subcortical level.

That the cortex may initiate affective pupillary reactions was shown by Karplus and Kreidl (1910). However, their viewpoint that this reaction is predominantly sympathetic will have to be revised in favor of central inhibition, as was their concept of the mechanism of reflex dilation (Ury and Gellhorn, 1939b). Our experiments also show that this reaction may be obtained from other cortical areas. This discrepancy may be due to absence of general narcosis in our experiments. The identity of these fields with those determined by S. Tower (1936) which give inhibition of extrapyramidal movement is suggestive of a common striatal factor, while the presence of a temporal field points to the affective potentialities of this area now being revealed by Kluver and Bucy (1939).

CONCLUSION

The threshold to pain reaction was studied on suitably trained, un-anesthetized cats after varying amounts of cortex had been removed. The indicator used was the reflex dilation of the pupil caused by inhibition of the Edinger-Westphal nucleus. Cortical areas giving this reaction were also determined. The results are as follows:

1. Ablation of the sensory or sensorimotor cortex does not raise the threshold to pain reaction.
2. Massive cortical lesions lower the threshold to pain reaction.
3. Cortical areas capable of inhibiting extrapyramidal movements also cause inhibition of the Edinger-Westphal nucleus.
4. The possible pathways of the indicator reaction are discussed.
5. A general scheme for pupillary activity is presented.

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POSTURAL REFLEXES AND GRASP PHENOMENA IN INFANTS

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INTRODUCTION

TONIC NECK REFLEXES and the various types of grasp phenomena (forced grasping, grasp reflexes of the hand and foot, volitional grasping movements) have been the subject of numerous experimental and clinical investigations. Tonic neck reflexes were studied and described in infants and children by Schaltenbrand (1925), Peiper and Isbert (1927), Gesell (1934), Byers (1938), and others. Grasp phenomena in infants have been described by Watson (1919), Givler (1921), Troemner (1928), Bryan (1930), Peiper (1931), Chaney and McGraw (1932), and Brain and Curran (1932). However, relatively few observations have been made regarding the possibility of any specific relationships between the tonic neck reflexes and grasping movements in normal and pathological human material.

MATERIAL AND METHODS

In order to study the relationships, if any, between tonic neck reflexes and grasp phenomena in normal humans, a series of observations were made in 70 healthy, newborn, premature and 30 healthy, newborn, full-term infants at the Kings County Hospital, Brooklyn, N Y. In addition, one case diagnosed clinically as amaurotic familial idiocy was studied. All infants whose birthweight was 5 lbs or less, were considered to be premature. The smallest infant examined weighed 2 lbs 13 oz but the usual weight in the premature group varied between 4 and 5 lbs. Most of the premature infants were examined on at least 2 different occasions and observed over periods varying from 2 weeks to 4 months. The full-term infants were examined only on one or two different occasions at some time during the first ten days of life.

At each examination, asymmetric tonic neck reflexes were studied and observations were made upon the concurrence of grasp phenomena. An asymmetric tonic neck reflex was considered positive when rotation of the head around its longitudinal axis with the infant initially in the normal symmetrical supine position produced relative extension of one or both limbs on the "chin side" of the body and corresponding flexion of one or both limbs on the opposite side. Rotation of the head was produced by the examiner's hands, which were placed as symmetrically as possible along both sides of the head. For purposes of photography a nursing bottle was presented to the infant's mouth after rotation of the head, and in this way a constant posture of the head was maintained without the struggling which at times accompanied the manual rotation. It is well recognized that in the young infant the act of sucking may be accompanied by strong flexion of the upper limbs. In our material the upper limbs often remained in or assumed an attitude of flexion with the hands partly or firmly clenched irrespective of whether manual rotation alone or manual rotation with subsequent administration of the nursing bottle was used. In general it was observed that the tonic neck responses were more definite, and more frequently obtained in the lower limbs than in the upper limbs.

* We are indebted to Dr George E Brockway, Chief of Department of Pediatrics, Kings County Hospital

OBSERVATIONS

Positive asymmetric tonic neck responses were obtained quite consistently in 64 of the 70 premature infants and in 16 of the 30 full-term infants during the course of an examination. In the remainder of the cases, the responses were variable and exhibited no constant postural pattern of the limbs upon rotation of the head through 180° of arc. In 9 of the 64 premature, and in 3 of the 15 full-term infants showing positive neck responses an initial flexion movement of the toes was fairly consistently observed in the flexed limbs while an initial extension movement of the toes was usually noted in the extended leg. In these cases, the flexion or extension attitude of the toes sometimes remained fixed in position so long as the relative flexion or extension attitude of the limbs persisted, thus appearing in the nature of a tonic response. Figures 1 and 2 illustrate flexion of toes in the flexed limb and relative extension of the toes in the extended limb. It should be noted that asymmetry in posture is definitely present in the legs as noted above, whereas the upper extremities remain fairly symmetrical in flexion. The maintenance of the position of strong flexion in the upper limbs was possibly enhanced by the presence of a sucking response. However, in this case the flexion was present before sucking was initiated. It should be noted, in addition, that the infant in Fig. 2 exhibited flexion of the toes as the right leg spontaneously flexed on rotation of the head, and the photograph was snapped as the infant rotated the leg medially. This eliminates the possibility of flexion of the toes as being associated with pressure on the sole of the foot. In Fig. 1, the relative flexion of the toes in the left foot is seen to exist with the leg in the lateral flexed position in which position pressure on the sole would not be a factor. In the remaining 55 premature and 11 full-term infants in the group showing the positive neck reflexes, a flexion-extension pattern of response of the toes was not consistently obtained. Nevertheless such responses occurred often enough to justify an interpretation that they were the dominant form of response. Other responses of the toes were also seen including at times flexion of the toes in the extended leg and extension of the toes in the flexed leg. Sometimes no changes in the positions of the toes occurred at all, even when the tonic neck reflex pattern of the limbs was very definitely positive. In some of these cases alternating flexion and extension movements of the toes in both the extended and flexed legs were often seen during the examination.

In order to study possible relationships between changes of the position of the body in space and concomitant grasp phenomena, all of the infants in the group were subjected to changes in position. The technique was to place the infant with one or the other side down either by placing the infant on its side on the bed, or by manually suspending it in the air, at the same time holding the head so that its normal symmetrical position relative to the body was maintained during these procedures. By this means, labyrinthine reflexes were prevented from changing the position of the head in relation to the rest of the body, thereby preventing any tonic neck reflexes



FIG. 1. (left) This figure illustrates the response commonly found in the group of premature infants on rotation of the head. It will be noted that with rotation of the chin to the right relative flexion of the left lower extremity and extension of the right lower extremity occurred. Associated with this, relative flexion of the toes in the left lower extremity and extension of the toes in the right lower extremity occurred. In this case asymmetry in the limb posture was not observed in the upper limbs, after rotation of the head and before the nipple was placed in the infant's mouth. The maintenance of the position of strong flexion in the upper limbs, however, was possibly enhanced by the presence of a sucking response.

FIG. 2 (right). In this case, upon rotation of the chin toward the left relative flexion of the right lower extremity and extension of the left lower extremity occurred. Simultaneously, flexion of the right toes and extension of the left toes occurred. Again, a relatively symmetrical flexed posture in the upper limbs was observed after rotation of the head, and before the nipple was placed in the infant's mouth. The maintenance of the position of strong flexion in the upper limbs, however, was possibly enhanced by the presence of a sucking response.

from coming into play. Thus, any digital flexion or flexion of the limbs which might occur as a result of changes of the entire body in relation to space could not be due to the influence of the tonic neck reflex mechanism. In the

whole group, however, no consistent pattern of response as regards flexion or extension movements in either the limbs, the toes or the fingers was obtained by this test. No definite relationships, therefore, were demonstrated in the newborn infant between the position of the body as a whole in space, and reflex postures in the limbs or toes.

In addition to the entire group of normal infants studied a case of decerebrate rigidity in a 19 month old Hebrew female child, diagnosed clinically as amaurotic familial idiocy was observed over a period of several weeks. This patient exhibited marked rigid extension of the limbs and spine with a "scissors attitude" of the legs and a position of the fingers and toes

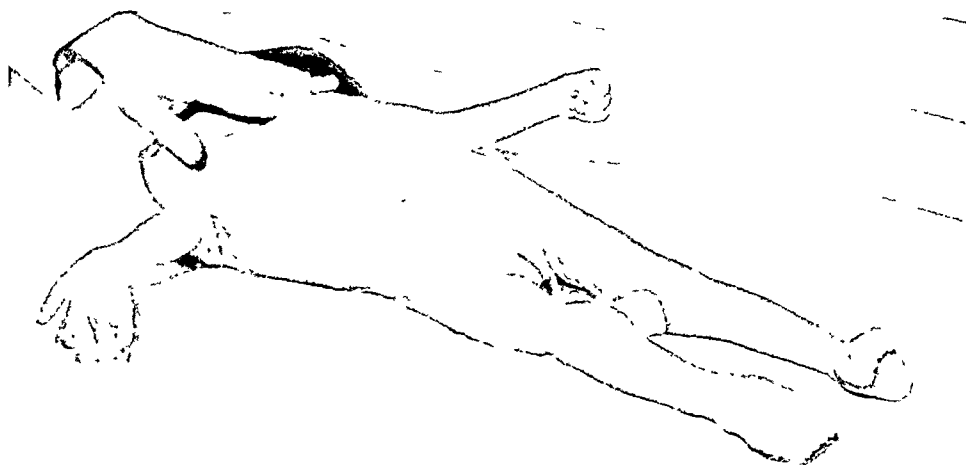


FIG. 3. This infant is clinically diagnosed as suffering from amaurotic familial idiocy. The usual spontaneous posture of the infant was that of decerebrate rigidity with hyperextension of all the limbs and opisthotonus. The constant response consisting of flexion of the toes opposite the "chin side" and the neutral position of the toes on the "chin side" is well demonstrated.

more or less midway between flexion and extension. This posture remained fairly constant with changes of the position of the entire body in relation to space. However, upon rotation of the head with the chin to the right, with the patient remaining in the supine position, a slow flexion of the toes on the left was observed, with practically no change in the position of the toes of the right foot (Fig. 3). When the head was returned to the mid-line position again the flexed toes on the left slowly returned to their original position, but as the rotation of the head was continued through a 90° angle to the left, gradual tonic flexion of the toes on the right occurred. They remained in flexion until the head was again rotated back to its original position. No change in the posture or the gross relative rigidity of the limbs, however, was obtained as the head was rotated from one side to the other. The fingers of the hand showed relatively little change in their positions or their tone during rotation of the head.

DISCUSSION

The above findings suggest the possibility of an interrelationship between the asymmetric tonic neck reflexes and the grasp phenomena of the toes. That this interrelationship is not primarily dependent upon mere flexion of the limbs alone is suggested by our observations. It appears, however, that there is more likelihood of flexion of the toes occurring in a limb which assumes the flexed position in response to rotation of the chin to the opposite side than in a limb which assumes or maintains an extended position under similar conditions.

The exact relationship existing between the tonic neck responses, the postural mechanism as a whole and the grasp phenomena, remains obscure. Fulton and Dow (1938) described postural neck reflexes in the labyrinthectomized monkey and their effects upon the grasp reflex, following total bilateral removal of areas 4 and 6 in the brain. In these operated monkeys they obtained release of the tonic neck reflexes and the grasp reflex, and found that any postural reaction originating from the neck or body that produces an increase of flexor posture in an extremity also causes intensification of the grasp reflex; any postural reaction causing an increase in extensor posture causes diminution in the grasp reflex. It was concluded, therefore, that grasping in primates is not an isolated reaction, but an integral part of the postural reflex mechanism. In an earlier publication, Kennard, Viets and Fulton (1934) had reported that in the human subject the strength of the grasp phenomena varies with the patient's position in space, exactly as described later for the monkey by Fulton (1934) and Bieber and Fulton (1938). The conclusion of Fulton and his coworkers that grasp is part of the righting reflex mechanism peculiar to primates was disputed by Walshe and Hunt (1936) who pointed out that it would be more accurate to say that grasping and the righting reflexes interact. They state that such interaction between reflexes of different physiological category is known to occur and in this connection mention as a case in point the influence of the tonic neck reflexes upon the Babinski response as recorded by Walshe (1923). In the study of their own cases of humans, however, Walshe and Hunt reported that they found no influence of body posture in relation to space or of head posture in relation to the trunk upon either grasping movements or upon reflex tonic grasping.

In most of the experimental material and in a great deal of the human material of other investigators, the experiments have in general dealt with either normal or pathological animal or pathological human cases. In our own material, consisting of normal human premature and full-term newborn infants and one pathological infant, we are led to believe that there is a definite effect of head posture in relation to the trunk upon grasping movements. In this latter respect our findings seem to agree somewhat with those of Fulton.

Our observations, in normal infants and in one pathological infant, concerning the relationships, if any, between grasp phenomena in the fingers

and toes and changes in the position of the body as a whole in space, however, do not seem to agree with those observed by Fulton and his coworkers for pathological animal preparations and pathological human material.

CONCLUSIONS

On the basis of our findings in human infant material, we feel justified in concluding that a relationship of some degree of concurrence exists between the tonic neck reflex mechanism and the grasp phenomenon, but that the latter is not necessarily an integral part of the tonic neck reflex mechanism. In addition, our material discloses no relationship of concurrence between changes of the position of the body as a whole in space and grasp phenomena.

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ACTION OF ETHER AND NEMBUTAL ON THE NERVOUS SYSTEM

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INTRODUCTION

EXPERIENCE has shown that potential records of activity in the central nervous system are too complex to permit easy analysis. This is a consequence of anatomical complexity which makes difficult the recording, except to a limited degree, of activity potentials of specific units. To determine the mode of action of an anesthetic on the nervous system it was decided to follow the plan, used in the study of strychnine action (Heinbecker and Bartley, 1939), of obtaining data on the effect of the agent on structures like the axon, the synapse, and the ganglion cells of the median nerve cord of *Limulus* heart which lend themselves readily to experimental attack. The results are utilized in an explanation of the more complex action of these agents on the central nervous system. While the extent to which it is permissible to interpret activities of the central nervous system in terms of the properties of peripheral axon, synapse and ganglion cell is still debatable, recent evidence indicates that comparable structures in the central and peripheral divisions of the nervous system act similarly qualitatively, but they may have different time relations.

The effects of ether and nembutal on the frog axon, on the turtle peripheral synapse, on the median nerve cord of the heart of *Limulus polyphemus*, on the spinal cord of turtle and on central nervous system of cat are here reported. It is assumed that activity in corresponding units of the nervous system of various animals will be qualitatively similar. Owing to the extent of the subject matter covered no attempt will be made to analyze the literature. Others, among them Derbyshire, Rempel, Forbes and Lambert (1936), have followed the action of anesthetics on the brain by a study of the potential changes they produce.

METHOD AND RESULTS

Effect of ether on form of axon potential The effect of ether on the form of the axon potential was investigated for the alpha group and also for single fibers. In one series of six experiments, potentials evoked by stimuli just above threshold for the alpha fibers were recorded from frog sciatic nerve 1 mm from the point of stimulation. The stimulating electrodes were connected into one arm of a Wheatstone bridge to eliminate large escapes of current into the leads. The cathode ray oscillograph in association with an amplifier was used as a recording mechanism. The potentials were made monophasic by crushing under the grid electrode and applying 2 per cent cocaine. Three per cent ether or 10 per cent nembutal dissolved in frog Ringer's solution was applied to the region of vertically placed recording electrodes by a drip method.

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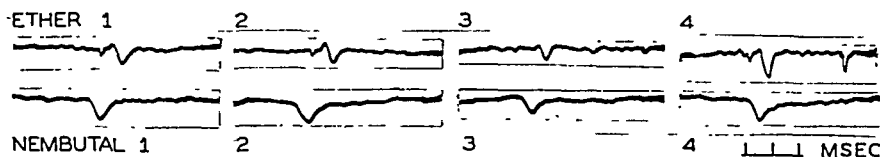


Fig. 1. Records of single axon potentials. 1 before application of the anesthetic. 2 and 3 after application of anesthetic. 4 after subsequent washing with Ringer's solution. Time in milliseconds. Note that there is no essential change in duration from the effects of ether or nembutal but in each instance a gradual diminution in area. On washing with Ringer's solution recovery is evidenced.

In all instances these changes lowered the spike potential of the alpha fibers with no increase in duration and with a decrease in area. After definite depression of the potential was evident, washing with Ringer's solution led to recovery of the potential. The negative after-potential is progressively lowered by ether and by nembutal.

In another series of six experiments, the effect of ether and of nembutal was studied in frog nerve on single axon responses made monophasic by crushing and cocainizing under the grid electrode. The preparation extended from the root region to the distal part of the tibial nerve. The stimulating electrodes were applied to the root and the recording electrodes to the fine distal part of the nerve. The drug was applied to the region of the recording electrodes. After definite depression of the potential was evident, washing with Ringer's solution was performed.

Ether and nembutal depress the amplitude of individual axon spikes without changing their duration other than is attributable to internodal delay (Fig. 1). Only A fibers were investigated but all our previous studies indicate that the other fibers would be similarly effected. Blair and Erlanger (1936) have reported similar findings for ether. They state that ether and cathodal polarization produce a block with the same general characteristics.

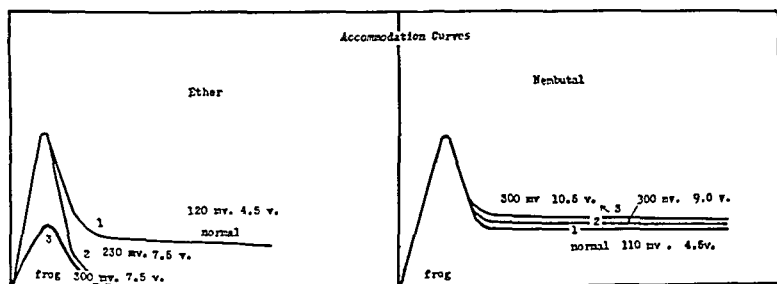


Fig. 2. A. Curves showing the relative amplitudes of nerve fiber responses to faradic shocks superimposed upon a subrheobasic cathodally polarizing current at various instants (abscissa) following its onset. The fall of the curves from their peaks is determined by the ratio and amount of accommodation. Curves of results of an experiment on a green frog sciatic nerve. In all curves MV indicates the voltage of the just subrheobasic current used; V, the voltage applied to the condenser (0.001 mfd.) for the shock stimulus. (1) Response height in millimeters at various instants after start of polarizing current for nerve before use of ether. R 120 μ V. V 4.5. (2) Response height after 2 minutes washing with 3 per cent in Ringer's solution. R 230 μ V. V 7.5. (3) Response height after 3 minutes further washing with ether. R 300 μ V. V 7.5. Interelectrode distance 9 mm. Conduction distance 15 mm. Silver-silver chloride electrodes used.

B. The curves of results of similar type of experiment on green frog sciatic nerve, 5 per cent nembutal in Ringer's solution used instead of ether. (1) Response height before application of nembutal. R 110 μ V. V 4.5. (2) Response height 11 minutes after application of nembutal. R 300 μ V. V 9.0. (3) Response height 9 minutes later. R 300 μ V. V 10.5. Note that ether increases accommodation, nembutal decreases it slightly.

Effect on fiber groups. The effect of ether and nembutal on nerve fiber groups was investigated in six turtle vagus nerves. This nerve yields, after conduction, recognizable A, B, and C potentials. The drugs were applied by the drip method to the region of the recording electrodes. Condenser discharges served as stimuli. It was found that the C fibers were first blocked, then the B fibers, and finally the A fibers. The A fibers recovered first, then the B fibers, and finally the C fibers.

Effect on electrical threshold, absolutely refractory period and conduction rate of fibers. The effect on electrical threshold and absolutely refractory period was determined on frog

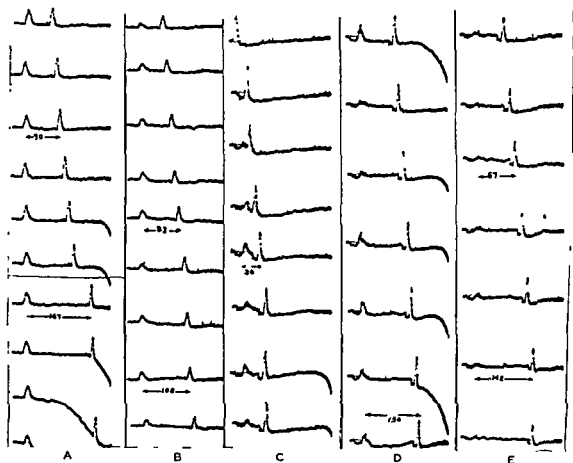


FIG. 3. Records showing the postganglionic responses from paired stimuli to the pre-ganglionic stretch of the superior cervical sympathetic ganglion of the turtle before and after the application of ether or of nembutal to the ganglion by the drip method. Both stimuli are submaximal for the B wave, the second being greater than the first. Column A, records before ether; B, after 3 per cent ether; C and D, records from another preparation before nembutal; E, after 3 per cent nembutal. The size of the response for the second shock varies as the interval of separation between the first and second is altered. Ether and nembutal shorten the period of recruitment as evidenced by the change in the interval of 70 to 167 msec. to 83 to 108 msec. and of 30 to 130 msec. to 67 to 142 msec., respectively, during which time the response to the second shock with paired stimulation is greater than the response to the second shock alone. Characteristically, recruitment begins earlier with ether than in the normal state and later with nembutal. The amount of facilitation under anesthesia is less than in the normal state.

nerves by applying ether or nembutal (5 per cent) to the stimulating electrodes; the effect on conduction rate of the alpha fibers by applying them to the nerve trunk between the stimulating and recording electrodes. From the onset of any recognizable effect of either anesthetic there occurred an elevation of the electrical threshold, a prolongation of the absolutely refractory period and a slowing of conduction. There was no evidence of any excitatory effect. Blair and Erlanger (1936) have presented evidence that the relatively refractory period of the axon is also prolonged by ether.

Effect on accommodation in nerve fiber. The effect of ether and nembutal on accommodation was investigated in green frog sciatic nerves. Only fresh nerves were used because it

was found that preparations soaked in Ringer's solution for several hours sometimes failed to accommodate to cathodal polarization to a normal degree. The drugs were applied in the region of the stimulating electrodes. In one series of 10 experiments a cathodally directed polarizing current, adjusted before each series of observations to be just subrheobasic in intensity and of 200 msec. duration, was applied once a second. A condenser shock ($0.001\mu\text{F}$. delivered through $35,000$ to $40,000\omega$ resistance), of a strength just adequate to yield a response 20 to 30 mm. in amplitude at the point of maximum effectiveness of the two stimuli, was superimposed through the same electrodes at various times during the flow of the polarizing current. The height of the response to the shock stimulus at the various intervals throughout the flow of the polarizing current was marked on translucent paper placed over the face of the oscillograph. This permitted a series of readings to be made very rapidly, thus eliminating as much as possible changes in the state of the nerve with time. By this method response height, not threshold, is measured for accommodation. While not ideal, because response height is an "S" curve against voltage, the error introduced is not great. Readings were made on both normal and anesthetized nerves.

Ether produced a definite increase in accommodation, nembutal a slight decrease (Fig. 3), and washing with Ringer's solution restored accommodation to normal. In another series of four experiments for each anesthetic agent, the strength of polarizing current used was that found just subrheobasic for the nerve in its normal state. This was done because it had previously been noted with strychnine (Heinbecker and Bartley, 1939) and it was again noted with ether that there is no constant relationship between a threshold change to a shock alone and a change in accommodation. With strychnine, a marked decrease in accommodation can be effected with the threshold for a condenser shock either lowered or raised. With ether, a marked increase in accommodation can be effected with no change in shock threshold. In normal nerve that the degree of accommodation increases with an increase in strength of the polarizing current (Blair and Erlanger, 1938). Since ether increases accommodation markedly, while nembutal decreases it slightly, the change in accommodation is not simply the effect of the polarizing current which is progressively elevated, to be kept just subrheobasic, as the shock threshold increases with increasing depression.

Effect on peripheral synapse

The excised superior cervical ganglion of the turtle was chosen for study of conduction through a peripheral synapse because it maintains relatively normal function for a considerable time after excision. It has a preganglionic stretch of 20 to 30 mm. and a long postganglionic one of 40 to 60 mm. Ether and nembutal (5 per cent) were applied by dripping onto the ganglion. Condenser discharges were used to stimulate and the electrical postganglionic responses recorded.

In six experiments with each anesthetic, conduction through the ganglion was progressively blocked. Because observation was continuous throughout the application of the agent, in effect all possible concentrations were observed. With stimuli placed two seconds or more apart there was never evidence of any increase in the submaximal potential area recorded from the postganglionic stretch. Ether blocked the ganglion in a few minutes and recovery was rapid on washing with Ringer's solution. Nembutal, in the concentration used, blocked the ganglion 5 to 10 times more slowly than ether and recovery from it was also much slower.

The same preparation served for studying latent addition (summation interval) and facilitation, as evidenced by recruitment. Paired brief stimuli, submaximal for the B wave but with the second always greater than the first, were applied to the preganglionic stretch and a monophasic record of the postganglionic responses secured. The maximum interval between

stimuli during which the area of the first and second response *together* was greater than the area of the second response alone measured the duration of latent addition; and the much longer interval during which the area of the second response when preceded by the first was greater than the area of the second response alone (recruitment) measured facilitation.

Latent addition averaged 2.2 to 2.5 msec. and facilitation was present from 50 or 70 msec. until 100 msec. Between the two there was a period of relative refractoriness or depression (Fig. 3). After the application of ether or nembutal (3 per cent) the period of latent addition was progressively shortened. By the time that the area of responses was depressed about 50 per cent, the period of latent addition was shortened approximately 50 per cent by ether and to a somewhat lesser degree by nembutal. The period of facilitation was markedly shortened by ether, to a lesser degree by nembutal. The amounts of latent addition and of facilitation are also lessened progressively by both drugs.

Effect on ganglion cells of median nerve cord of Limulus polyphemus. In 20 preparations, ether and nembutal (5 per cent) were applied to the isolated nerve cord of *Limulus*, or to the nerve cord left *in situ* on the excised active heart, or to the nerve cord *in situ* in the body after removal of a narrow strip of the carapace above it. In the later two types of preparation the effects observed are considered to represent primarily the action of the anesthetic agents on the nerve tissue and not on the heart muscle. *Limulus's* heart is neurogenic, and the validity of the above assumption is indicated by the fact that direct electrical stimulation of the heart muscle was still effective after application of the anesthetics to the nerve cord and surrounding heart muscle had abolished spontaneous activity. Further, the potential derived from the nerve cord precedes appreciably the muscle potential. The duration of the potential of the nerve elements varies directly with the duration of the contraction.

Ether, in 6 good excised preparations, increased the frequency of the ganglionic discharges, indicated by heart rate and the areas of the potentials associated with nerve and muscle responses (Fig. 4). In some preparations *in situ* the heart rate decreased for a brief period and then, with slightly increasing anesthetization, it increased. This increase persisted until the potential area associated with each burst of activity fell practically to zero. Similar results were obtained when the heart was left *in situ*.

With nembutal (Fig. 4) there was at times an initial acceleration followed characteristically by a slowing. Associated with the slowing there was an increase in responsiveness, indicated by the potential area, when the heart was left *in situ* and with a maintained blood supply; but such an increase was usually not observed in excised preparations. With still deeper anesthesia the usual and typical effect was a decreased frequency of ganglion cell discharges, *i.e.*, of heart rate, with a gradual decrease in area of the potentials associated with nerve and muscle activity. When the normal coordinated rhythm of the system had broken down, isolated units could be seen to discharge separately at similar frequencies.

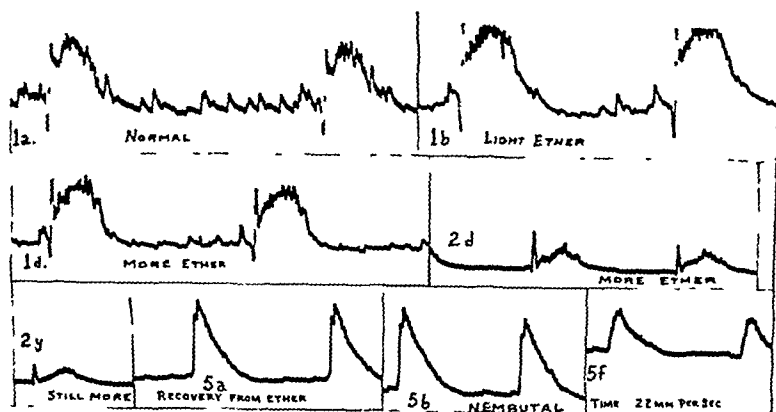


FIG. 4. Records of potentials from the excised median nerve cord and muscle of the heart of *Limulus*. One needle electrode placed in the tissue close to the median nerve cord, the other at a distance of 2.5 cm. on killed muscle. 1a, normal response; 1b, 1d, 2d, 2y, responses after application of 3 per cent ether in sea water by dripping it onto the median nerve cord. Note in 1b an increase in size of the response with increase in heart rate. 1d, 2d, and 2y show progressive diminution in the size of the response but the increase in rate becomes progressively greater. 5a, the response after recovery from ether by washing with sea water. 5b and 5f, the responses after application of 5 per cent nembutal. Note in 5b a slight increase in the size of the first response after nembutal with an increase in the frequency of the heart rate. In 5f, the typical slowing of the heart with a diminution in response is shown. Time—22 mm. per sec.

Recovery from ether was readily effected with Ringer's solution with a reversal of the depression effects. Recovery from nembutal was always much slower and frequently not complete during one to two hours of observation.

Effect on spinal cord. The effect of these agents on spinal cord activity in the turtle was studied by recording the reflex response of a muscle to a fixed stimulus. In 8 preparations the cord was exposed and transected at the junction of the middle with the distal third. The sciatic nerve on one side was exposed and stimulated with a current just strong enough to yield a good crossed flexion response, the magnitude of the contraction being

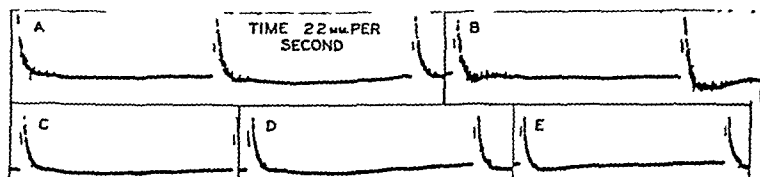


FIG. 5. Records of response from the left quadriceps muscle of the turtle using needle electrodes on stimulation of the right sciatic nerve at 4 second intervals. Cord transected at junction of middle with distal third. Blood supply probably not greatly interfered with. A, normal response. B, response after application of 3 per cent ether in frog Ringer's solution to cord surfaces. Note the increase in number and frequency of the individual responses and the increase in size of the contraction. C, D and E, records after further application of ether. Note decrease in number and frequency of the individual responses with a decrease in the size of the contraction.

measured with 2 needle electrodes inserted into an active thigh muscle. Ether (5 per cent) and nembutal (5 per cent) were applied to the exposed cord below the level of section.

Ether (Fig. 5) led, first, to an increase in reflex activity, as evidenced by increased frequency of individual potentials and increased total area of the electromyogram. This phase was followed by a decrease in frequency and in total area. Nembutal sometimes produced a slight transient increase in frequency and total area of the muscle potentials, though never so marked as with ether, and then regularly led to depression. Washing away the ether

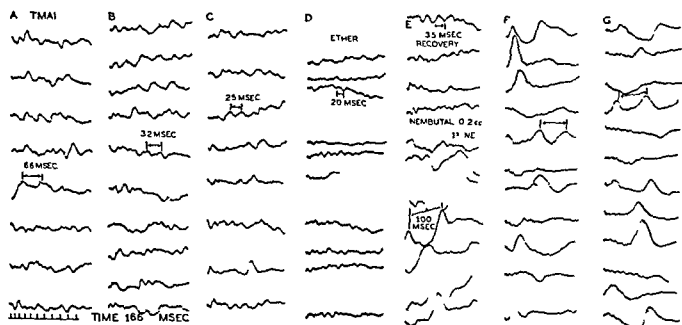


FIG 6 Record showing the spontaneous activity of the sensorimotor cortex under 3 conditions A, normal, B, with 3 cc of ether injected into airline, C, with additional 3 cc of ether, D, with 4 more cc ether. Upper records of E after partial recovery from ether, lower records after a first injection of 0.2 cc nembutal (1 per cent) F, somewhat later G, still later. Although the waves exhibited are of several frequencies one type of wave can be picked out and followed in the different states of the animal. In A the period between such wave crests is about 50 msec, in B, 32 msec, in C, 25 msec, in D, 20 msec. Recovery in E returns the period to 35 msec. Nembutal quickly shifts the period to about 80 to 100 msec. Time 166 msec.

led to fairly complete recovery, in 5 to 15 min, recovery from nembutal was slow and still incomplete after 1 to 2 hours.

Effect on cortical potentials. The effect of ether, used as a general anesthetic, on the activity of the sensorimotor cortex is shown in Fig. 6. There is a definite increase in the frequency of the normal waves. Occasionally the area of the potentials is slightly increased at the very onset of anesthesia. Nembutal anesthesia slows the wave frequency and at first increases the wave area, with frequent spindle formation (Fig. 6 and 7). With increasing concentrations of either drug the area of cortical potentials is gradually decreased.

In the early stage of ether anesthesia, the electrogram, recorded from electrodes placed deeply in the mid portion of the thalamus is increased and

this increase persists when the cortical activity is already becoming depressed. With deeper anesthesia the thalamic potentials are also decreased. This early increase cannot be regarded entirely as a release phenomenon due to cortical depression, because it occurs on both sides of the thalamus even when one cortical hemisphere has been removed before the start of anesthesia. Under light nembutal anesthesia, there is an increase in area and a decrease in frequency of potentials from the thalamus and basal ganglia.

The immediate cortical response to stimulation of the isolated saphenous nerve is a series of waves in the sensory area (Fig. 8 and 9; ether or nembutal anesthesia). When needle electrodes are used, with grid superficial and

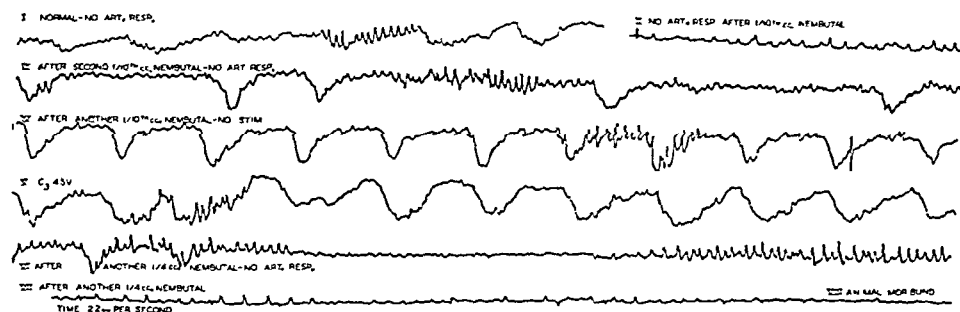


FIG. 7. Records of potential activity of sensori-motor cortex after tetra-methyl-ammonium iodide (designated as normal) to show the effect of progressively increasing nembutal anesthesia. Records IV and V show large waves which have the frequency of the artificial pulmonary ventilation. They may be artefacts but such large waves are at times the result of afferent stimuli developed by the respiratory movements. Record V shows no effect from strong stimulation of the saphenous nerve once every two seconds even though the cortical waves are well sustained. Note the progressive lowering and slowing of the alpha waves especially in record VII & VIII. Time 22 mm. per sec.

ground lead 2 to 3 mm. deep, the rising phase of the first wave (α_c) is surface positive and reaches a crest 8 to 10 msec. after the shock to the nerve. Its negative phase fuses with the second wave (δ_c) which is negative and has a crest time of 40 to 50 msec. A third positive wave follows with a crest time of 120 to 140 msec. When stimulus to the saphenous is strong enough to excite the C fibers, there follows a third negative wave (C_c), with a crest time of 400 to 500 msec., and a positive wave with a crest time of 1100 to 1200 msec. (Fig. 9). Assuming a conduction distance of 0.5 meter, and a synaptic delay of a few msec., the crest times of the α_c , δ_c , and C_c waves are such as would result from conduction in fibers at rates about 80, 20, and 1.2 meters per sec., respectively. These values definitely associate the cortical waves with the alpha, delta (B_1) and C fibers of the cat's saphenous nerve (Heinbecker, Bishop and O'Leary, 1936).

The origin of the potentials which constitute the immediate response cannot be definitely stated. The duration of the waves is consistent with the interpretation that they represent summated fiber potentials. If so, their

quick depression by light anesthesia at a time when spontaneously active elements in the cortex are little effected would indicate that the afferent fiber pathways below the cortex are quickly blocked. If they represent, as well, potentials of cortical interneuron elements, then the latter probably constitute by far the major portion of them.

Gradually deepening ether or nembutal anesthesia depresses slowly the amplitude and area of the three waves. The third wave is depressed most rapidly, the second somewhat later, and the first wave last. Under light surgical anesthesia the third and second waves are gone while the first remains one-half to one-third its normal size. As these late waves are depressed, there is a coincidental elimination of the more general changes in cortical activity which normally follow stimulation of the saphenous nerve, especially of its slower fibers. The findings fit well with the known order of dis-

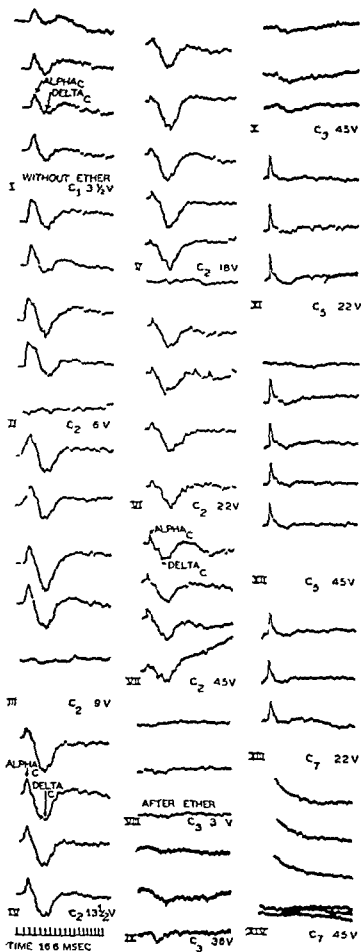


FIG. 8. I-VII, records of the earlier waves of the immediate response of the sensorimotor cortex to increasing strengths of condenser shocks applied to the saphenous nerve. Note a short first surface positive wave, α_c , with a crest 8 to 10 msec. from the start, followed by a surface negative wave with a crest at 40 to 50 msec., δ_c . This wave is probably superimposed upon the negative portion of the first wave. A third surface positive wave with a crest at about 150 msec. is seen. Records VIII to XIV show the effect of ether on this portion of the immediate response. Note the depression of the waves most marked on the late part of the response and least on its early part. Due to time

relationship after the shock, the α_c and δ_c waves are in whole or in part regarded as at least the expressions of activity in afferent fibers corresponding to those giving rise to the α and δ waves of the conducted saphenous nerve potential. C_1 0.001; C_2 0.005; C_3 0.01; C_4 0.005; C_7 0.1 μ F. Time 16.6 msec.

appearance of sensibility under ether anesthesia, pain sense being lost first and touch and pressure last.

Effect on facilitation in central nervous system

Facilitation was determined in the cat under the influence of tetra-methyl-ammonium iodide because anesthesia was found to depress it. Two

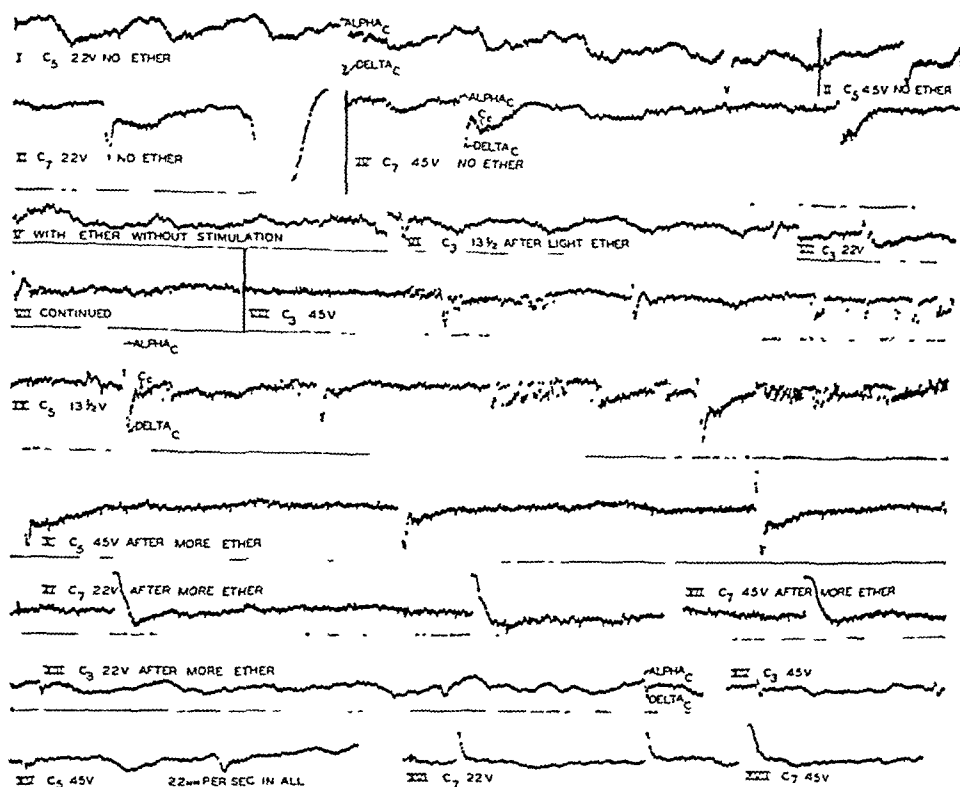


FIG. 9. Records of potentials from sensori-motor cortex to show the immediate response to saphenous nerve stimulation and the effect of ether on it when condenser shocks adequate to stimulate C fibers are used. C₃ 13.5 V just above thresholds, C₇ 45 V supra-maximal for the C wave. Note the increase in area of the immediate response with increasing shocks in records I, III, and IV. In record IV the first wave, alpha_c referred to in Fig. 8, is not recognizable except as a slight brief upswing of the line and is mixed with the shock escape. Its crest time is 8 to 10 msec. Wave delta_c has a crest at 40 to 50 μ after the shock and wave C_c at 400 to 500 msec. The values are what would be expected from fiber pathways having an approximate velocity from 80, 20, and 12 meters per sec. if a conduction distance of 0.5 meter and a few sigmas for synapse time are assumed. These are reasonable figures for the speed of the cat saphenous alpha, delta, and C waves. The effect of ether is shown in record VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, & XVI. It depresses the third wave most, the second somewhat less, and the first wave least of all.

nearly equal submaximal stimuli at varying separation were applied to the saphenous nerve (giving alpha plus delta waves) once every 2 sec. and the immediate cortical responses recorded from the sensorimotor area. At a

separation interval so great that the period of relative refractoriness of the direct fiber pathway could not play a role, the size of the second response was increased if preceded by the first stimulus. The second response area is difficult to observe accurately until the stimulus interval is about 100 msec., then it increases gradually to a maximum at about 200 msec. The area of the facilitated second response is greater than that of the second response alone for a period of 80 to 100 msec. (period of facilitation as evidenced by recruitment) (Fig. 10). Spontaneous variations in form and magnitude of the general cortical potentials and a rhythmical rise and fall in the magnitude of the immediate response make it difficult to define with accuracy the amount and the duration of the period of recruitment or to

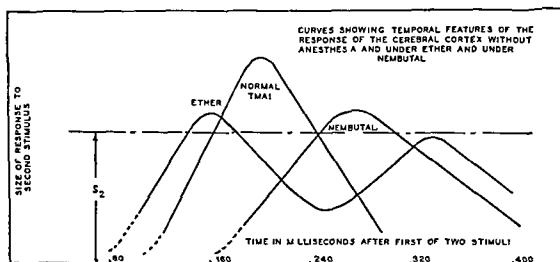


FIG 10 Curves depicting the size of the cortical response from the sensori-motor area to the second of two nearly equal stimuli to the saphenous nerve submaximal for the whole nerve trunk, nearly maximal for the alpha and delta waves under tetra-methyl-ammonium iodide (TMAI in figure), under light ether and light nembutal anesthetics. S indicates the relative maximal size of the response to the second stimulus alone during the excitability cycle. Note that the second response is visible earliest under ether and latest under nembutal, indicating an earlier recovery of excitability under the former. The amount of facilitation as measured by recruitment is greatest under tetra-methyl-ammonium iodide. It is lessened by ether and nembutal. Time in msec

observe accurately the instant at which a second response appears. Under tetra-methyl-ammonium iodide increasing ether anesthesia progressively shortens the period of recruitment and decreases its amount; nembutal shortens it less and decreases its magnitude less. The second response is observable earlier under ether than under tetra-methyl-ammonium iodide and later under nembutal. It is of interest to note that the crest times of the periods of recruitment under tetra-methyl-ammonium iodide, ether, and nembutal, 200, 150, and 250 msec. respectively, indicate that facilitation is developed on a cycle of excitability similar to that which determines the frequency of the alpha rhythm. The alpha frequency under these same drugs is 5, 6, and 4 per sec., respectively. The site at which facilitation occurs is not localized by these experiments; probably it is in the nerve cells of the afferent pathway and the first cortical interneuron cells.

In good preparations, without anesthesia, stronger stimuli applied to the saphenous nerve at regular intervals of 0.5 to 0.3 sec. lead to increase in the magnitude of successive immediate cortical responses. This may be regarded perhaps as another type of facilitation. It is associated with a widespread increase in cortical activity, hence it is probably not an expression of events limited to a particular unitary system within the nervous system. Ether and nembutal depress this type of facilitation rapidly.

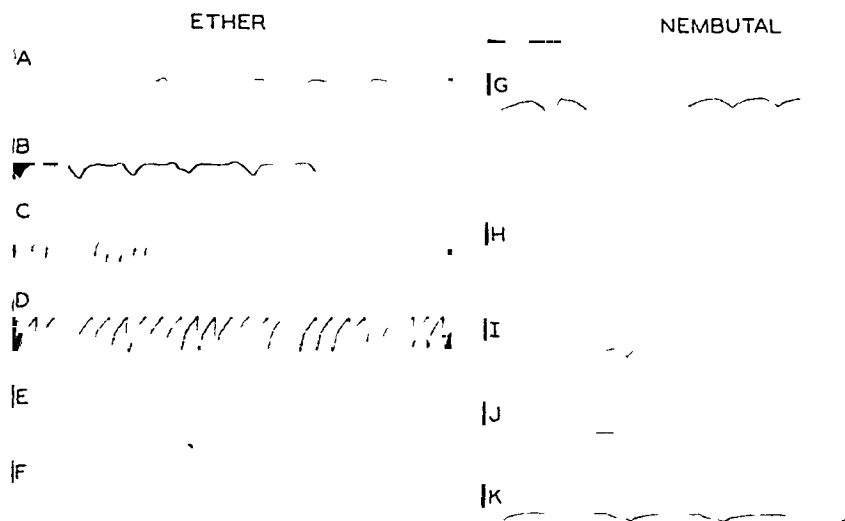


FIG. 11. Chest pneumograms to show the effect on respiration of 10 per cent ether in normal saline administered intravenously to a normal dog. A, normal; B, C, D, E, records showing chest movements under progressively deepening anesthesia. In B is noted a slowing of respiratory rate. It is considered to be an expression of release of the respiratory center from afferent vagus influences. C and D show the excitatory effects of ether. In E anesthesia is very deep but there is no slowing of respiration. F shows the effects of some degree of asphyxia. G, record of another normal dog. H, I, J, and K, records to show effect of 10 per cent nembutal administered intravenously. Note in H an increase in the rate and depth of respiration. I, shows a lessening of these effects. In J and K, slowing of respiration is well established. Note the prolongation of inspiration. Downstroke inspiration.

The existence of a cycle of excitability in the cortex was first demonstrated by Bishop (1933) when he noted that shocks of constant value elicited a variable cortical response unless the rate of stimulation was properly adjusted. This rate in the rabbit was some multiple of 5 per sec., the frequency of the alpha rhythm for this animal. Bartley (1936), using paired maximal stimuli to the optic nerve, plotted the size of the cortical response to the second shock as the interval between shocks was varied. When they were close together (but beyond 20 msec.) the response to the second was absent, with increasing separation it increased in size to a maximum at an interval of 180 to 200 msec., the period of the alpha rhythm under the conditions of the experiment.

Effect on phrenic discharge. Cats were vagotomized and the distal part of a phrenic nerve freed for potential recording under local anesthesia with tetra-methyl-ammonium iodide and artificial ventilation. The animal was then slowly anesthetized with ether or nembutal and changes in the phrenic discharge followed. In dogs the respiratory movements were recorded with a pneumograph applied around the chest and the anesthetic was administered in 10 per cent concentration in Ringer's solution intravenously. It was thus possible to record the typical action of the respiratory mechanism of the intact animal and the effect on it of these anesthetic agents.

Ether (Fig. 11) first increased the frequency and amplitude of the response, sometimes after a transitory slowing without much change in amplitude. The increase in rate persisted as inspiration was gradually depressed by deepening anesthesia. Only in the terminal stages was the respiratory rate slowed. Nembutal (Fig. 10) led to a transitory increase in the frequency of respiration with a slight increase in the depth of inspiration, which was soon followed by marked slowing of respiration and depression and slowing of inspiration which progressed with depth of anesthesia.

Phrenic nerve potentials showed similar changes in the frequency of discharge and the duration of the discharge process. The increase in amplitude of inspiration during the excitatory stages was associated with an increase in frequency of the individual potentials comprising the total potential record, and slowing of inspiration with a decrease. It has been previously shown that the discharge lasts throughout inspiration (Heinbecker, 1937) and that the individual nerve fibers to inspiratory muscles are active approximately throughout this period (Bronk and Ferguson, 1935).

The results show a striking difference in effect of the two anesthetic agents on the respiratory center; ether increases the frequency of its discharge while nembutal decreases it. Ether, during light anesthesia, is much more an excitant than is nembutal.

ANALYSIS OF RESULTS

Before interpreting the potential changes in the central nervous system resulting from ether and nembutal, it is necessary to state our views based on the work of this laboratory concerning the nature of synaptic and nerve cell activity and as to the source of the potentials recorded from the cortex and spinal cord.

In the median nerve cord of *Limulus polyphemus* a long sustained simple potential (Heinbecker, 1933), on which are superimposed small individual potentials having the duration of axon spikes, is associated with activity in a large pace-maker ganglion cell. Potentials of this type were recorded from short masses of ganglionic tissue in which only a single pace-maker cell was shown to be present by the examination of serial sections of the tissue while preparations without such cells failed to yield the large long potentials. The pace-maker function or autochthonous rhythmicity of these ganglion cells has been established beyond question by Carlson (1909),

Hoffmann (1911), and Heinbecker (1933). Further, the duration of the period of activity of the fibers and frequency of the individual axon spikes varied directly with the amplitude and duration of the long sustained potentials with which they were associated. The cell response can be modified both as to rate and magnitude; and, other conditions remaining the same, the potential area decreases inversely with the frequency.

On the basis of such evidence from *Limulus* it is inferred that spontaneously rhythmic nerve cells in the mammalian cortex are mainly responsible for the long sustained potentials observed in the electroencephalogram. Similar long potentials recorded from the spinal cord after extrinsic nerve stimulation are interpreted as due to the prolonged depolarization of interneuron and anterior horn cells produced by synaptic stimulation. This view is shared by Eccles (1939). Nerve cells of the type found in the superior cervical sympathetic ganglion do not give rise to long potentials of any appreciable magnitude. Their activity would then not be expected to be associated with repetitive axon discharges. That they are not, was early established by Heinbecker (1930) in the turtle and by Bishop and Heinbecker (1932) in the cat.

Blair and Erlanger (1939) demonstrated that the action potential of a fiber can be made to stimulate across a non-responding gap of one or two internodal segments. It seems reasonable to assume that the fiber action potential could stimulate across the gap of a dendritic or cell body type of synapse in a similar manner. The effectiveness of synapse stimulation would then depend upon the strength of the axon action potential, the electrical conductivity of the synapse medium, and the electrical excitability of the structure beyond the synapse. The available evidence indicates that the effects of the axon action potential across the synapse are at least two, one of brief duration measurable as the period of latent addition, and a second of much longer duration (maximum at 200σ for the A fibers in the sensorimotor region of the cat and 100σ for the B fibers in the cervical sympathetic ganglion of the turtle) measurable as the period of recruitment. In the nerve fiber both processes have been intensively studied, the first as the period of latent addition, the second as the period of super-normality. The period of recruitment here measured has the time characteristics of the slow after-potential recorded from the sympathetic ganglion by Eccles (1937). The period of facilitation for the central nervous system now reported has time values more like those of after-potentials than of spike potentials. We suggest that the potential recorded from the *Limulus* ganglion cell is associated with a process similar to the one responsible for the sympathetic ganglion cell negative after-potential, the magnitude and duration of which are reflected in the magnitude and duration of facilitation.

Inhibition of the nerve cells by ether and by nembutal is similar to inhibition of the pace-maker ganglion cells of the heart of *Limulus* by extrinsic inhibitory nerves, at least in that both result in a depression of that potential assumed to be associated with the cell response. In this prepara-

tion, acetylcholine depresses the potentials much as does stimulation of the extrinsic inhibitory nerve fibers. Ether, which increases accommodation in the axon shortens the cell response, while strychnine which decreases accommodation in the axon lengthens the cell response (Heinbecker and Bartley, 1939). Nembutal, which decreases accommodation to a slight extent, acts like strychnine before it depresses appreciably. To support a suggestion that nerve cell inhibition may be effected through a process similar to that resulting in increased accommodation in the axon is the demonstration by Gilson (1939) that stimulation of the turtle *vagus* increases accommodation of the heart muscle and markedly shortens its response. It is possible that accommodation is an expression of the chemical reactions of the "activity or response substance" on which an excitatory or inhibitory substance acts; for we have shown that there is no constant relationship between a change in initial shock threshold and a change in accommodation.

Some workers have suggested that inhibition, as such, may be associated with the development of a positive potential. No evidence of this has been found during studies of the *Limulus* heart cord in depression. A reversal in potentials does occur but is always due to an unequal depression at the vicinity of the recording electrodes. The cyclic change in cortical excitability and responsiveness is, however, associated with potential variations.

The actions of ether and of nembutal on the various preparations used indicate that depression of the intact nervous system probably is the result of a depression of activity in axons and in the dendrites and somata with which they synapse. These drugs depress the early and late excitatory processes and the response process in both fiber and cell. Depression of the axon is shown by a lowering of the axon spike potential and the negative after potential. The electrical threshold of a nerve trunk is raised, the absolutely and relatively refractory periods of its fibers are prolonged, and the conduction rate is slowed. The smallest fibers are depressed before the large ones. Depression of the cell excitatory process is indicated by reduction in the amount and duration of latent addition and of facilitation; and of cell responsiveness by a decrease of the slow potentials associated with their response and by a diminution or cessation of activity in their associated axons.

The relative susceptibility to depression of the axons, dendrites, and cells comprising the central nervous system differs. Our results seem to warrant the interpretation that activation through the synapse is eliminated before the responsiveness of the cell beyond is greatly depressed. Spontaneous activity in the cortical cells, for example, is well sustained long after the immediate response to saphenous nerve stimulation is entirely eliminated. Blocking at the synapse might then be the result of a lowering of the action potentials in the fine axon terminations which impinge on the nerve cell or its dendrites. Indirect evidence for this is seen in the slowing of respiration just after ether is administered intravenously in the intact

animal for this change would follow the elimination of afferent vagus impulses. Ether *per se* increases the frequency of response of isolated spontaneously active nerve cell complexes.

Depression of all synapses by anesthetics does not proceed equally throughout the nervous system. It was previously noted by Bishop, Heinbecker and O'Leary (1934) that the ability of afferent vagus stimulation to modify blood pressure was much less affected by ether anesthesia than was its ability to accelerate respiration. Synapses of effector mechanisms (bouton type?) such as those concerned in respiration are relatively unaffected by concentrations of anesthetic agents which completely block synapses of afferent pathways. Cortical potentials are almost completely depressed by ether or nembutal before respiration ceases. The speed of anesthetic action also varies in different regions. It is most rapid in the cortex, slower in the spinal cord, still slower in the basal ganglia and brain stem, and slowest in the peripheral portions of the sympathetic and somatic nervous systems.

Our results indicate that ether, as a depressing agent, modifies the properties of the nerve axon in a manner similar to cathodal polarization (Blair, 1938). The axon spike potential is lowered, the period of latent addition is shortened, the absolute and relative refractory periods are prolonged, and accommodation is increased by both. Conversely, strychnine (Heinbecker and Bartley, 1939), as an excitant, changes the properties of the axon in the same way as does anodal polarization (Blair, 1938).

We have here no evidence concerning particular chemical changes resulting from the action of ether or of nembutal on the nervous system, but presumably these drugs depress the chemical reactions involved in both the excitation and the response processes.

SUMMARY AND CONCLUSIONS

The actions of ether and nembutal on the nerve axon, the synapse, the rhythmically active nerve cell, the spinal cord, and the brain have been analyzed principally in terms of the action potential changes they produce.

The amplitude and area of the axon spike potential and the negative after-potential are progressively diminished by ether and by nembutal.

The electrical threshold of the axon is raised, its conduction rate is lessened, and its absolutely refractory period is prolonged by ether and by nembutal.

Ether markedly increases accommodation in the axon, nembutal decreases it slightly.

Both ether and nembutal block conduction through a peripheral ganglion. This is interpreted as indicating that they progressively lessen excitation of the soma through the synapse.

With depression by ether or nembutal, the period of latent addition (summation interval) at the synapse is shortened and the effectiveness of temporal summation is lessened. Further, the period of recruitment is shortened by these agents in proportion as they decrease the magnitude of

the cyclic variations in amplitude and area of the immediate cortical response

Ether increases the frequency of discharge of nerve cells possessing spontaneous rhythmicity and nembutal decreases it, often without any initial increase. Both drugs first increase the magnitude of the response of nerve cells and then, with increasing anesthesia, decrease it. The increase produced by nembutal is considered to be in part a consequence of the reduction of the response frequency.

Ether and nembutal differentially depress the immediate cortical response to saphenous nerve stimulation, the order of final extinction of its constituent waves being third, second, and first. The general cortical excitation produced by spread is eliminated as the late waves are depressed. These effects are interpreted as due to blocking of synaptic stimulation.

The action of ether on the nerve cell or cell group suggests that it first stimulates and then depresses the excitation and response mechanisms. Nembutal has a much feeblere preliminary stimulating action. The duration of the cell response under ether is less than that under nembutal, and recovery of responsiveness under ether is more rapid than under nembutal. This may account for the different discharge frequencies induced by these agents.

Present and past researches of this laboratory bearing on the question of excitation and inhibition of neurons are analyzed. The facts suggest that excitation is an effect on the soma produced by the summation of brief electrical stimuli which arrive at the terminals of impinging nerve axons. A brief and a prolonged excitatory state develop in the soma following their action. Inhibition, aside from the well known rhythmical changes in responsiveness, has not been found to be accompanied by the development of a potential. It results in a lowering or elimination of the potentials associated with activity and presumably would oppose the activation of nerve cells.

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DEGENERATION AND REGENERATION OF SYMPATHETIC SYNAPSES

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INTRODUCTION

THE SUPERIOR cervical sympathetic ganglion is an easily accessible part of the nervous system in which to study the form and function of simple synaptic junctions, for as was shown by Bishop and Heinbecker (1932), all impulses passing in by the preganglionic fibers must traverse a synapse before emerging by the postganglionic fibers. Eccles (1936) has stimulated the postganglionic trunk electrically and has been unable to record any transmitted impulse in the preganglionic fibers. He found that all preganglionic impulses suffered delay in passing through the ganglion and concluded that all preganglionic fibers end in synapses on the cells of the superior cervical ganglion. In the present study, histological examinations have been made of the synaptic endings of the normal, degenerating, and regenerating fibers entering the ganglion. These findings have been correlated with the results of parallel electrophysiological observations on these synapses. The histological studies were made with silver stains, and the electrical studies with a cathode-ray oscillograph.

The literature which bears directly on the present work falls into the following three categories: (i) The structure of normal sympathetic synapses. (ii) Experimental degeneration of sympathetic synapses. (iii) Experimental regeneration of sympathetic synapses.

Normal sympathetic synapses. The papers of Smirnow (1890), Cajal (1891, 1893, 1903, 1905), Huber (1899), and Ranson and Billingsley (1918) contain the early and fundamental work on the structure of the synapses in the sympathetic nervous system of normal animals. De Castro in 1923, and Lawrentjew in 1924, contributed further studies and during the past fifteen years they with their associates have brought forward a wealth of information concerning sympathetic synapses.

De Castro (1923) used the silver staining technique of Cajal for blocks of tissue fixed in pyridine and ammoniacal alcohol, and the frozen section method of Cajal (1921) for mammalian ganglia fixed in formalin. His preparations showed the preganglionic fibers approaching the ganglion cells and breaking up into fine fibrils which insinuated themselves between the dendrites and their overlying capsule and finally ended in delicate rings on the perikaryon. Lawrentjew (1924) using a modification of the Golgi staining method found similar structures in the superior cervical ganglion in normal cats and dogs.

Fedorow and Matwejewa (1935) used the Gros-Bielschowsky technique to demonstrate sympathetic synapses in the frog. Preganglionic fibers were

seen to wind spirally around the dendrites of the ganglion cells and to form one or more rings or discs en route, usually at the junction of the dendrite with the cell body. Thereafter the fibril ramified in many different ways, ending in filamentous loops or terminal discs which possibly represented the "growth cones" of the developing preganglionic fibers. Fedorow (1935) has obtained convincing cinematographs of the pericellular terminations of preganglionic sympathetic axones in living preparations of frogs. Insinuated between the nerve cell and its connective tissue capsule minute discs were visualized attached to fine spirally arranged preganglionic fibrils. The capsule was draped over the crater-like hole of the bouton ring as a piece of cheesecloth is placed over the mouth of a churn. Electrical stimulation of the preganglionic fibers *in vivo* caused these boutons to assume brilliant coloration when neutral red was present as an indicator. In the same laboratory Smitten (1937) confirmed the subcapsular position of the synaptic endings, and showed that when the capsule was stretched with micro-dissecting needles the ring-like boutons under it took the form of ellipsoids.

Stöhr (1935) rejected the neurone theory as applied to the sympathetic nervous system and claimed that there is no interruption of the fiber pathway at the synapse. Nonidez (1937), using stains specific for connective tissue, has been able to stain selectively the "periterminal reticulum" which Stöhr believed to be nervous in nature. Kolosow and Polykarpowa (1935) have shown the presence of this reticulum after preganglionic sympathetic fibers have been destroyed. Lawrentjew (1939) succeeded in reproducing Stöhr's pictures of the "periterminalreticulum" only after immersing fresh material in 30 to 40 per cent formol for fixation. Stöhr and his pupils offered no physiological evidence in support of their thesis, nor have they carried out the elementary degeneration experiments which would prove or disprove the nervous composition of their "periterminalreticulum." The writer, after examining Stöhr's preparations in Bonn, was unconvinced of the nervous nature of the structure there demonstrated as the "periterminalreticulum."

Degeneration of synapses. Waller (1851), Budge (1855), Nikolajew (1893), Tuckett (1896) and Langley and Anderson (1896) were the first to study experimental degeneration in preganglionic fibers and synapses. Waller as early as 1852 remarked upon the "disorganized" state of preganglionic fibers 6 days after they had been transected. Ranson and Billingsley (1918) using the pyridine silver stain, were unable to demonstrate the normally dense intercellular plexus in the superior cervical ganglion of cats once the preganglionic trunk had been divided and had degenerated.

De Castro (1930) carried out a comprehensive research on degeneration in the superior cervical ganglion in more than one hundred cats. He used the block methods of silver impregnation after fixation of young material in hypnotics such as somnifene and chloral hydrate. Seventeen hours after dividing the preganglionic fibers he detected throughout their length, early fragmentation with granular changes, and an increased affinity for silver salts. At 24 hr. the terminals were granular and hypertrophied, and by 36 hr. only a few remained. At 5 days a few resistant fibers could be seen interlacing among the cells, but at the end of the 7th day all had broken down into heavy dark granules. In the 24- and 36-hr. cats a medium shock from an induction coil caused a slight dilation of the pupil. After 48 hr. a strong stimulus failed to evoke any response.

Lawrentjew (1934) used the Gros-Bielschowsky technique in his study of degeneration and regeneration in the superior cervical ganglion in 78 animals, mostly cats. His histological findings and his series of mechanical recordings from the nictitating membrane confirmed the work of De Castro.

Regeneration of synapses. Waller (1853), Schiff (1894), Langley (1897, 1900) and Tsukaguchi (1916), have demonstrated functional regeneration in the sympathetic nervous system at varying periods of time following suture of the divided ends of the preganglionic trunk. De Castro (1930) observed the severed preganglionic fibers regenerating in the cervical trunk within 24 to 72 hours, throwing out a brush of short branches, each ending in a spherical "boule de croissance" or a ring-like "bouton de croissance." Regenerating fibers entered the ganglion within 5 to 12 days depending upon the degree of injury and the distance of the lesion from the ganglion. Eight days after compression or 12 days after division of the cervical trunk close to the ganglion, subcapsular boutons were visible on both perikarya and dendrites of the sympathetic neurones. From 15 to 17 days after division, a medium stimulus caused changes in the pupil and nictitating membrane, which accorded with the arrival of the earliest bouton endings on the ganglion cells. Forty to 50 days after operation many of the ganglia had complete functional and structural restoration.

Lawrentjew (1934) reported progressive stages in regeneration similar to those in De Castro's series, and compared the "growth bulbs" on the regenerating fibers to the spherical endings on the axones of neurones in tissue cultures which Lebedew and Sorin had demonstrated in his laboratory.

MATERIAL AND METHODS

Cats ranging in weight from 1.4 to 3.8 kg. were anaesthetised with Nembutal intraperitoneally. A single incision was made on the left side of the neck to suit the later arrangement of electrodes in the oscillograph room. On reaching the carotid sheath great care was taken in the longitudinal separation of the preganglionic sympathetic trunk and its artery from the vagus nerve. In the average animal the preganglionic trunk was transected 2.5 cm. below the superior cervical ganglion to ensure a sufficient portion of regenerated trunk from which to "lead off" electrically at a later date. In the degeneration studies the preganglionic trunk central to the point of division was dissected out and removed as far down in the thorax as possible, to prevent any confusing regeneration. In the cases in which regeneration was to be studied great care was taken in the approximation of the freshly cut ends of the sympathetic trunk. Two Cushing silver clips were applied longitudinally to the sheaths of the nerve segments, and served to keep the junction clean as well as to make it easy to locate the exact point of section later on.

Electrophysiological recordings were made on the animals following decerebration under deep ether anaesthesia. The cathode ray oscillograph technique (Eccles, 1935a), was used, the animals being kept in a humid chamber throughout.

The superior cervical sympathetic ganglion and its appended pre- and postganglionic trunks were fixed in 15 per cent formalin (Merck) after the blood vessels had been washed through with warm Ringer solution followed by 5 per cent formalin in Ringer. The addition of 2 to 3 drops of pure pyridine (Kahlbaum) per 10 cc. of fixation formalin improved the quality of the staining in the Cajal and Río-Hortega methods. The optimum fixation time was from 10 days to one month, but sometimes much older material proved satisfactory. In general, the younger animals gave the best histological pictures. When it was desired to store an excess of frozen sections already cut, these were placed in a 5 per cent solution of formalin, and then washed well before using.

After trying many methods for blocks and for frozen sections the following were selected as being the most useful for staining sympathetic boutons:—Río-Hortega's double impregnation method for neurofibrils (1921); Cajal's modification for frozen sections (1921); and the Gros-Bielschowsky method as demonstrated by Fedorow in Lawrentjew's laboratory in 1937. All these techniques and notes concerning their vagaries and peculiarities have been detailed by the author (Gibson, 1937, p. 481).

EXPERIMENTAL RESULTS

Normal superior cervical sympathetic ganglion. Histological examination of the normal superior cervical sympathetic ganglion disclosed synaptic structures similar to those existing in the central nervous system. The two usual forms, boutons terminaux and boutons de passage could be clearly seen. Their number and distribution were, however, very different from those in the central nervous system. The largest number of boutons, of whatever type, seen on one cell and its processes, was thirteen. One microscopic field yielded twenty boutons on focussing, but in the best stained sections the maximum was twenty boutons per hundred cells.

The terminal boutons (Fig. 1) were darkly stained with clear smooth outlines. Some were so large (6μ to 7μ) that they could not be seen completely in one focal plane. The average endings were 2μ to 3μ in diameter. Both circular and angular types were usually seen to be continuous with a very fine fibril. Dendrites as well as perikarya showed boutons in contact with their surface.

Boutons de passage (Fig. 2) were present in normal ganglia in much greater numbers than were boutons terminaux. On the results obtained with the present stains it would seem possible that the boutons de passage are the more important synaptic structures. They occur only on the finest preganglionic fibers and are not to be seen unless these delicate, ramifying filaments have been impregnated. Boutons de passage are definite, darkly stained rings which occur along the course of preganglionic fibers at varying distances from their termination. They enable one fiber to make contact with many different ganglionic cells or even with many different points on one cell. As many as 5 such contacts around the edge of one perikaryon have been seen on focussing.

Normal boutons de passage rarely exceed 2μ in diameter, and on the average they are considerably smaller. In silver slides counterstained with methylene blue, boutons de passage were subcapsular, making direct contact with the perikaryon. Some have been seen as near as 3μ to their terminal bouton. The boutons de passage vary in shape from circular to elliptical, but in the latter case they are always symmetrically tapered.

On a few cells of one normal ganglion some minute, platelet-like structures were clearly visible. They seemed to be confined to the surface of the cells, were elliptical in shape and darkly staining. They had light, though not hollow centers. No neurofibrillar connections could be seen after repeated examinations under the oil-immersion lens. They did not resemble any sort of connective tissue structure; they may represent an abnormal arrangement of the chondrioma of the ganglion cells as described by Ortiz-Picon and Perez Lista (1929).

Degeneration of preganglionic fibers. The earliest observations on de-

generation were made 2 days after dividing the preganglionic trunk 2.5 cm. below the superior cervical ganglion. The preganglionic fibers were deformed and shrivelled, and had an increased silver affinity. The myelin sheath along many fibers was disintegrated into coarse granules. The boutons terminaux were slightly swollen and darkly stained. In some cases the fibril leading to the bouton was not appreciably changed, and in one case a fiber was seen intact after its granular bouton had broken off. Boutons de passage increased slightly in size, and in density of staining.

Two days after operation the action potential spike was normal in shape although a little diminished in size in comparison with that of the ganglion of the unoperated side. This probably indicated that fewer ganglion cells were discharging impulses. There was no significant slowing of the impulse conduction in the preganglionic fibers. The latent period of response to a stimulus was a little longer than normal, possibly indicating a slight lengthening of synaptic delay. As can be seen from Fig. 9a, the S_1 and S_2 components of the spike could be separated.

By the 3rd day of degeneration, destructive changes were further advanced. Some fibers had become fenestrated and hypertrophied, while the heavier boutons were still easily distinguished from swollen or vacuolated fibers. At four and one-half days the resistant fibers were affected over their entire course, and filling in of the rings was seen (Fig. 3). No neurofibrillar changes were seen inside the ganglion cells. Six days after division, the preganglionic fibers were either broken down into granules engulfed in Schwann cells, or were in a swollen and fragmenting state. The fine intercellular plexus of fibers had disappeared, and only a few residual boutons could be seen (Fig. 4).

The preganglionic fibers were inexcitable just distal to the point of section. Nearer the ganglion a few were still excitable, but their threshold was very high. A small discharge was set up from the ganglion, but the spike action potential was only about 2 per cent of normal. It was definitely due to synaptic transmission to the ganglion cells for it was abolished by painting nicotine (1:1000) on the ganglion. The diminished size of the spike shows that the synaptic excitation of only a few ganglion cells was adequate to excite the discharge of impulses. The synaptic delay was 7 to 8 msec., at the shortest, and as long as 15 msec. for some of the impulses transmitted. It was not possible to be sure what group of ganglion cells was still functioning (Fig. 9b).

After 8 days, only granular debris could be seen in and around the Schwann cells where the disintegrated fibers entered the ganglion. No fine fibrils were observed at this stage. Strong electrical stimuli on the preganglionic trunk failed to produce any action potentials in the ganglion cells or in the postganglionic fibers. However, antidromic impulses backfired into the cells via the postganglionic fibers produced normal action currents from the cells (cf. Eccles, 1936). *Thus the ganglion cells themselves were not affected by degeneration of the preganglionic fibers.*

Regeneration of preganglionic fibers. The first signs of regeneration were seen 11 days after the preganglionic fibers had been divided. Thin unmyelinated fibers bearing small loop endings could be seen in the peripheral stump of the sutured preganglionic trunk. These were sharply defined by the silver staining and tunnelled along the old courses between the Schwann cells.

By 14 days there was no recovery of function, and no electrical response of the ganglion cells or postganglionic fibers was elicited by preganglionic stimulation. At 21 days heavy fibers could be seen in the preganglionic trunk at the entrance to the ganglion, but no action potential of the ganglion cells or postganglionic fibers could be recorded on stimulation.

Regeneration of boutons was first seen 44 days after operation. The fine terminal fibrils which had been present in the normal ganglia had returned and had made contact with cells and dendrites after running a sinuous course. Many of the incoming fibers were myelinated distal to the neuroma and into the ganglion itself. Small boutons terminaux and boutons de passage stained darkly and were applied to the dendrites or to the cell body (Fig. 5). No neurofibrillar changes could be seen inside the ganglion cells.

A strong electrical stimulus to the preganglionic trunk between the point of section and the ganglion itself, produced a double spike action potential in the postganglionic fibers. The first spike was very large, and the second was well-formed but small (Fig. 9c, d). A strong stimulus just central to the nerve suture caused a fairly large first action potential spike followed by a small later one. A weak stimulus caused only a single response of medium height (Fig. 10a, b). A strong stimulus applied 31 mm. below the neuroma

FIG. 6 Terminal bouton on a regenerated fibre making contact with a sympathetic ganglion cell 70 day cat. Río-Hortega stain

FIG. 7 A fine regenerated pre-ganglionic fibre ending by means of a terminal bouton on a cell in the superior cervical ganglion 92 days after cutting the pre-ganglionic trunk. A bouton de passage is indicated at the opposite end of the cell. Río-Hortega technique

FIG. 8 Four figures by Lawrentjew to show the effect of concentrated fixation formol on nervous tissue. The upper two indicate, from left to right, the clear-cut nerve fibres after fixation in 12 and 20 per cent formol respectively. The lower left panel shows the "periterminalreticulum" appearing with 30 per cent formol, while the right shows the reticulum produced by fixing in 40 per cent formol

FIG. 9 (a) Action potential spike recorded from the superior cervical ganglion 2 days after its pre-ganglionic trunk was divided. The S_1 and S_2 components are visible. (b) Greatly diminished spike (2 per cent of normal) after 6 days' degeneration. Highly amplified. (c) Regeneration in 44-day cat. Action potentials recorded when the pre-ganglionic trunk was strongly stimulated 31 mm. central to the point of division. (d) A weaker stimulus produced a single spike

FIG. 10 (a) 44-day cat. Action potentials recorded after stimulation of the pre-ganglionic trunk 8 mm. central to the ganglion, and distal to the neuroma. A strong stimulus produced a large spike followed by a small late one. (b) A weak stimulus at the same point as in (a). (c) 44-day cat. Stimuli were applied 17 mm. central to the ganglion (t_e , just below the suture). Two spikes were recorded after a strong stimulus. (d) A weak stimulus failed to evoke the later spike. (e) 92-day cat. Regeneration of the pre-ganglionic fibres. A strong stimulus produced an S_1 spike followed by an S_2 wave. (f) 92-day cat. A weak stimulus produced only the S_1 spike, but the wave remained negative for a considerable period, suggesting a small discharge from S_2 cells.

evoked two large spikes, whereas a weaker stimulus brought out only the first spike (Fig. 10c, d).

The preganglionic trunk central to the point of section conducted impulses at almost normal rates. The part peripheral to the suture, however, had a conduction rate of 1.5 m. per sec. for the fastest fibers, and 1.1 m. per sec. for the average fibers; *i.e.*, less than 10 per cent of normal values. Allowing for this slow preganglionic conduction time the synaptic delay at the fastest ganglion cells (presumably S_1) was not more than 4 msec., which is only a little more than the normal, *i.e.*, 3 msec. The delayed spike which appeared on strengthening the stimulus to the preganglionic fibers was presumably due to the connection made by a group of high threshold regenerated fibers with a group of ganglion cells having a very long synaptic delay. In this case the synaptic delay was as long as 20 msec., and was probably due to S_4 cells.

With a still stronger preganglionic stimulus, either to the undegenerated or to the regenerated sections of the preganglionic fibers, a still later spike appeared in the ganglionic action potential. This was probably due to an even later response of some ganglion cells. Thus, the preganglionic fibers of higher threshold regenerated so as to evoke responses from ganglion cells having longer synaptic delays. This strongly suggests that there has been selective regeneration of the fast, low-threshold preganglionic fibers to cells with short synaptic delays, and of slow, high-threshold fibers to slow cells. The other possibility is that the higher threshold fibers take a longer time to set up the discharge of impulses from whatever ganglion cells they make synaptic contact with in regeneration. That is, after regeneration, a long synaptic delay may not be a true indication of a slow fiber making contact with a slow cell.

In the 70-day cat the regenerating fibers could be seen entering the ganglion after a very tortuous course through connective tissue. The majority of the fibers were unmyelinated and ran in groups of 5 to 10. Fibers branched into fine fibrils which made contact with ganglion cells and their dendrites by means of boutons de passage and boutons terminaux (Fig. 6).

Electrical studies showed an increase in the speed of conduction in the regenerated preganglionic fibers. The records indicated an intermediate stage between the incomplete regeneration in the 44-day cat and the full regeneration in the 92-day cat. The ganglion of the 92-day cat showed the restoration of the fine intercellular plexus. Many of the thinnest fibrils could be traced to the surface of ganglion cells where boutons de passage not more than 1μ in diameter could be seen. Most fibers were as yet unmyelinated. Figure 7 shows both a bouton terminal and a bouton de passage on a large ganglion cell.

The conduction rates in the regenerated preganglionic fibers ranged up to 7 m. per sec. for the S_1 fibers and to about 2 m. per sec. for the S_2 fibers (these are from $\frac{1}{3}$ to $\frac{1}{5}$ of the normal values). The threshold of the fibers was less than in the 44-day and 70-day cats, but was still about twice the normal value. The synaptic delays were 3 msec. for the S_1 fibres and 6 msec. for the

S₂ fibers, approximately normal values (cf. Therman, Forbes and Galambos, 1940). The ganglion cells showed normal inhibition and facilitation responses (cf. Eccles, 1935b). Again there was good evidence that the high-threshold fibers had made contact with the ganglion cells with the longest synaptic delays, and the low-threshold fibers with the ganglion cells with the shortest synaptic delays. There were probably some exceptional fibers, however, since a weak stimulus applied to the preganglionic trunk produced both the large spike potential and a small late negative wave, which might have been due to S₂ cells discharging. A strong stimulus brought out S₁ and S₂ spikes very clearly. (Fig. 10 e,f).

DISCUSSION

The normal action potentials recorded after antidromic stimulation in cases where all preganglionic fibers had degenerated, confirms the histological evidence that sympathetic ganglion cells are not affected by the loss, through degeneration, of the synaptic endings of preganglionic fibers. Also, the ganglion cells show the normal responses of facilitation and inhibition as soon as the regenerating fibers make contact with them. The synaptic delay of the ganglion cells is the same at the beginning of regeneration as it was before degeneration, showing that the regenerated synapses, from the start, closely resemble normal synapses.

Degeneration in the sympathetic nervous system has been shown to follow the course of that in the central nervous system (cf. Hoff, 1932, and Gibson, 1937), but it is somewhat slower.

A certain amount of histological confirmation of the division of the ganglion cells into S₁, S₂, S₃ and S₄ types has been brought by Solovieva (1937) who has described at least three distinct groups, on the basis of the ratio of cell area to nuclear area.

There has been much speculation whether the finest fibrils can transmit impulses to the ganglion cells by a number of non-specialised contacts. On the basis of the present results it might be said that the boutons shown were not numerous enough to be considered the only synaptic structures. However, the staining methods at present available make it impossible to say with any certainty that all the boutons present were stained.* It cannot be too strongly emphasized that no evidence whatever has been found to support the claim of Stöhr and his pupils that there is protoplasmic continuity across sympathetic synapses. Everything observed in this work has been in favor of discontinuity.

Finally, the imperfections of silver staining make it necessary for any investigator to leave room for possible non-specialised contacts as synapses. The history of the discovery of boutons in other parts of the nervous system in numbers sufficient to account for synaptic transmission, suggests that the boutons in the sympathetic nervous system may eventually be shown to be the specific synaptic agents.

* Improved staining techniques are now in process of development by Prados (1939) and Leach (1939), the former using vitamin C as a reducer in the Cajal techniques for blocks, and the latter using a special haematoxylin stain for boutons.

SUMMARY

A series of experiments is recorded in which the progressive stages in the degeneration and regeneration of synapses in the superior cervical sympathetic ganglion were studied histologically by silver stains, and physiologically by the cathode-ray oscillograph. Transmission through the ganglion diminished with the progressive experimental degeneration of the boutons attached to the preganglionic fibers. Restoration of normal function in the ganglion returned when the regenerating preganglionic fibers re-established contact with the ganglion cells by means of boutons de passage and boutons terminaux. Evidence is presented which strongly suggests selective regeneration of S_1 fibers to S_1 cells, etc. No histological or electrical evidence was found which would indicate that the ganglion cells suffered in any way from the degeneration of the synaptic contacts on their surface. The experimental results confirm unequivocally the theory of structural discontinuity in the sympathetic nervous system.

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STUDIES OF MOTOR PERFORMANCE AFTER PARIETAL ABLATIONS IN MONKEYS*

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INTRODUCTION

IN MAN and even in the inarticulate monkey, *motor* paralysis may be to some extent differentiated from paralysis of *sensation*, but it is difficult in either form completely to distinguish the purely motor symptoms from the changes in motor performance which result from interruption of the afferent portion of the reflex arc. Thus a limb may for a time hang motionless and flaccid when either an afferent or an efferent spinal root is severed, and it is only through detailed analyses of the syndrome that one may differentiate the underlying causes. The effects on motor performance of cortical ablation involving more complex sensory levels of integration are more difficult to analyze since the parietal cortex contains motor Betz cells (Betz 2, Levin, and Bradford 10); furthermore, the motor areas receive direct thalamo-cortical projections (Polyak 11); and hence the cortical motor and sensory areas cannot be anatomically separated. Physiologically the sensorimotor cortex has been described as a unit by Dusser de Barenne (4).

The sensory defects which follow lesions of the parietal cortex have been described in man (Head 8) and monkeys (Ruch 12), and are known to be due to changes in both the tactile and proprioceptive elements of sensation. Alterations in motor performance also appear, and such "ataxia" has been described in man by Head (8), Guthrie (7), and others. In the present paper an attempt has been made to study the alterations in *motor* performance which ensue in the monkey after pure parietal lesions and to compare them with those which follow lesions of areas 4 and 6 of the precentral cortex.

METHOD

In 11 monkeys (8 *M. mulatta* and 3 mangabeys [2 *Cercocebus torquatus atys* and 1 *C. galeritus chrysogaster*]) unilateral and bilateral ablations were carried out involving part or, all of the parietal lobes. In one animal the depths of the central sulcus were incised to sever transcortical U-fibers; in two others complete deafferentation of a single extremity was followed by a contralateral postcentral cortical ablation. General behavior, neurological status and particularly motor hand performance were studied before and after operation.

OBSERVATIONS

The principal observations are covered in the following illustrative protocols:

EXPERIMENT 1. *Seriatim* ablation of left areas 3-1-2; right areas 3-1-2; left areas 5 and 7; right areas 5 and 7. Transient dysmetria, ataxia; permanent hypotonia and marked bilateral atrophy, knee jerks hyperactive. Placing and hopping responses abolished [P.C. I].

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This animal was a normal immature sooty mangabey male weighing 1.8 kg. Hand performance previous to operation was described as follows: "Either hand is stretched forward for small bits of food. Fingers reach somewhat beyond and as soon as the palmar aspect of the fingers touches the food they are flexed and the food scraped toward the palm where it is held. If the particle is solid like a fragment of peanut, the thumb is put into play with a partial opponens function against the action of the middle and index fingers."

First operation—Left postcentral gyrus (Apr. 22, 1938). The left postcentral gyrus (areas 3, 1, 2, Fig. 1) was removed, dissecting out all tissue to the depth of the central sulcus. On the following day there was no definite paresis on the right side. The right hand and foot were held more in extension than the left. They appeared limp and showed no trace of the associated movements of the normal limbs. Walking and climbing were performed well with some overreaching of the right extremities. Food was approached with the left hand and brought to the mouth. Then the right hand was also placed on the food and thus assisted in feeding."

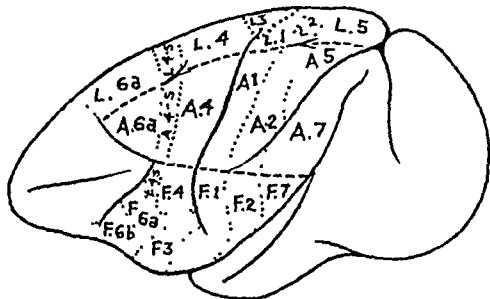


FIG. 1. Map of areas of monkey's sensory cortex (*Macaca mulatta*) (After Dusser de Barenne, J. G., McCulloch, W. S., and Ogawa, T. *J. Neurophysiol.*, 1938, 1: 436-441).

During the next two weeks the left hand was always used in preference to the right. When the right was used it appeared weaker and although able to grasp and pick up small objects, had less clearly defined movements of the fingers. Locomotion and ordinary cage activity appeared normal except for occasional exaggeration of movements of the right arm and leg in stepping. Resistance to passive manipulation in the right extremities was diminished and the knee jerk was pendulous. There was no atrophy. Placing and hopping reactions were absent in the right lower extremity.

Second operation—Right postcentral gyrus (May 18, 1938). The right postcentral gyrus was removed (areas 3, 1, 2). Again care was taken to extirpate to the depth of the central sulcus. One day after operation the animal fed itself using both hands, the right hand now showed greater dexterity than the left and, probably because it was now the more skilful of the two, it was used more and better than before the second operation. "In walking and climbing the left extremities hand and foot were everted slightly. Movements were quick and surprisingly accurate. In picking up food there was slight hesitation and the animal would look at the food for 2 or 3 seconds before initiating movement." There was a tendency to hold the left arm and leg extended. They appeared flaccid and sometimes dragged behind in walking.

One week later both hands were used thus. "For small bits of food the thumb is dextrously adducted against the side of the index finger. The monkey inverts its hand and then using mainly lips, tongue and teeth manages to get the food into its mouth." A wild grab was the most usual method of procuring food and there was never a true use of the opponens function, i.e. apposition of thumb pad to index or middle finger pads. A week later (May 31) posture was normal except for slight eversion of the hands and feet. "The animal

jumped, climbed and grasped showing no difficulty. When offered peanuts it reached through the bars picking them up and shelled them using both hands." Knee jerks were hyperactive.

Third operation—Left posterior parietal region (July 7, 1938): The remaining portions of the left parietal lobe (areas 5 and 7) were then ablated. Following this there was an additional deficit in motor performance, chiefly a tendency to exaggerate movements in walking and climbing. Often the right extremity became "lost" or tangled in the bars or chain. Resistance to passive manipulation was slight on either side but less on the right than on the left. Placing and hopping were bilaterally absent. The animal fatigued easily and atrophy of thigh muscles began to be noticed. From this time until the fourth operation the right fingers were used less than the left. On both sides vision was used noticeably in guiding the fingers. Food was grabbed and not deftly picked up. It was often dropped. The right hand was more affected than the left. Later although placing and hopping reactions were absent on the right, hopping and proprioceptive placing could be produced with strong stimuli on the left.

Fourth operation—Right posterior parietal lobule (Nov. 16, 1938): On the day after removal of the right areas 5 and 7 resistance to passive manipulation was equally reduced on both sides. Knee jerks were active and pendulous. Placing and hopping responses were absent bilaterally. Cage behavior was cautious and the animal remained on the floor supported on a broad base. Sensory deficit was very evident and in attempting to pick up food it groped and pulled at its own toes until it saw what was happening. The right hand was used in preference for feeding. No precise finger movements were seen. There was a fine tremor. Often the fingers became twisted with each other and at times the hand could not be extended through the wires of the cage without getting caught in various ways. This was true of either hand but the left was less accurate than the right.

By December 14 extreme wasting of all muscle groups in all extremities was noted. Knee jerks became increasingly active while hypotonia persisted. During the next 6 months there was little improvement. Ordinary cage activity was performed normally with only a slight eversion of the hands and feet. Fingers and toes were extremely plastic, atrophy was very great and fatigue easily induced. Loss of postural sense was obvious as bizarre postures and accidental striking and hitting against objects occurred constantly. Placing and hopping reactions remained absent, fine finger movements were grossly awkward.

EXPERIMENT 2. *Seriatum* ablation of left areas 3-1-2; right areas 3-1-2; left areas 5 and 7; right areas 5 and 7. Contralateral transient hypotonia. Eversion of hands and feet, permanent awkwardness in grooming; abolition of placing and hopping responses [P.C. II].

The subject, a mature female "brown mangabey" (*Cercopithecus galeries chrysogaster*) was particularly fitted for observation as it was tame and fond of "grooming," a procedure in which fine movements of fingers and thumbs are necessary (Fig. 2). "The picking finger, always the index, is kept almost straight and the others are semiflexed. When a scale is loosened it is picked up between thumb and first finger. A hair is also held in similar fashion between thumb pad and index pad."

FIG. 2. Fine prehensile finger movements of "grooming" in a mangabey (Exp. 2) following bilateral removal of parietal lobes.

First operation—Left postcentral gyrus (May 26, 1938): The left postcentral gyrus was removed (areas 3-1-2), care being taken not to injure the rostral lip of the central sulcus. One day later the animal would pick up objects with the right hand but although power was good it tended to use the fingers either too strenuously or too softly. It used its



mouth often rather than attempt thumb and finger in apposition. Slight hypotonia especially of the fingers was noticed. Three weeks later use of the right fingers was still awkward. That of the left continued accurate. No other deviations from the normal were noticed.

Second operation—Right postcentral gyrus (June 15, 1938) The right areas 3, 1, 2 were then extirpated, following which it was noted that the left extremities hung limp and that tactile placing was bilaterally absent. In picking with the left index finger, the animal used random full movements of the arm instead of discrete movements at the phalangeal joint. The amount of pressure was not controlled and often in grooming the fingers bit too deeply. Attempts to put either hand between the bars of the cage were defeated many times by inability to hit the opening. Posture, both while static and in motion, was normal.

During the next four months there was some recovery of precision in grooming. Resistance to passive manipulation became normal.

Third operation—Left posterior parietal lobule (Nov. 23, 1938) The left areas 5 and 7 were then removed. Immediately an added deficit appeared in the fingers of the right hand although in all other cage performance there was no change noticed. In grooming it usually reaches for my arm with its left hand and holds it very accurately. It then begins to move the right hand in an attempt to groom but either catches its fingers on the bars of the cage or scratches them along my arm. Very soon it becomes disgusted and then uses the left hand in accurate grooming movements.

Fourth operation—Right posterior parietal region (Dec. 16, 1938) The remaining posterior parietal region (areas 5 and 7) was then removed from the right hemisphere. During the first day after operation no attempt was made to groom with either hand. During succeeding weeks, however, it resumed this occupation (Fig. 2). In April, 1939 posture was normal except for slight eversion of the hands and feet. Placing and hopping responses were bilaterally absent. In grooming all fingers were used instead of the thumb and index finger only. Fine prehension was absent and the intensity of pressure of the fingers was very variable and not well controlled. The deep reflexes were active, resistance to passive manipulation seemed normal.

EXPERIMENT 3 *Ablation of left areas 1-2, 5, 7. Right transient hypotonia, weakness and proprioceptive loss. permanent tactile loss. Ablation of right posterior parietal area. Transient left hypotonia and depression of knee jerks, fine finger movements poorly done, ultimate deficit greater on right than left* [P. C. VI]

First operation The left parietal lobe (1-2, 5 and 7) was removed from a rhesus monkey (Oct. 12, 1938) with the exception of area 3, the posterior lip of the central sulcus which was left untouched so as to be certain that no precentral tissue was inadvertently damaged. The animal then showed transient depression of the knee jerks and limp extremities together with preference for using the normal hand in all fine manipulations. Placing and hopping reactions were at first absent, later hopping returned.

Second operation—Right areas 5 and 7 (Dec. 2, 1938) The right posterior parietal region only was then removed. The right hand immediately thereafter became used temporarily in preference. Tactile placing was absent on both sides, hopping absent on the left.

On the 8th day the use of fingers was still too coarse to permit feeding with small objects unless vision was used to correct movement. Resistance to passive manipulation was less in the left leg than in the right. Eventually, the greater deficit in all these motor acts appeared on the right extremity opposite the larger lesion. Hypotonia was greatest on the right. The left hand was used in preference and its manipulations were better than were those of the right hand. The normal smoothness of prehension was absent being replaced by jerkiness and occasional tremor. But the fingers of the left hand by January 1, 1939 were able to pick up objects by apposition of thumb and index finger. Hopping responses then became bilaterally present. Tactile placing returned on the left but not on the right.

EXPERIMENT 4 *Section of transcortical fibers between left areas 4 and 3. transient hemiparesis. Recovery. Ablation of left parietal lobe. Reappearance of slight deficit in right extremities, especially fingers* [P. C. V]

First operation—Section of left U fibers (June 14, 1938) Fibers in the depth of the central sulcus were sectioned through its length thus dividing connecting fibers between

areas 3 and 4. A transient hemiparesis resulted characteristic of area 4 ablations. Knee jerks on the right became pendulous, placing and hopping responses disappeared but later returned. Recovery of motor function also appeared complete before the second operation.

Second operation—Left parietal lobe (Dec. 9, 1938): After removal of the left parietal lobe in its entirety there followed immediately a postural defect characterized by pendulous dangling of the right arm and leg during locomotion. Placing and hopping responses disappeared on the right and remained permanently absent. Resistance to passive manipulation did not seem diminished but the right knee jerk became more pendulous and the right extremity showed eventually slight atrophy.

The fingers of the right hand were never used by preference for those of the left. All movements of the right fingers were, however, quickly accomplished and there was very little deficit save in the finest digital movements. Inaccuracy in gauging distance and strength could then be observed.

From the above observations and those made on 7 additional animals, it is evident that the motor deficit which follows parietal lesions is specific and can be clearly distinguished from the syndromes which follow ablation of area 4 or 6. Unilateral removal of areas 3-1-2 or of 1-2 alone caused identical changes, and removal of areas 5 and 7, changes which were similar in quality, but less in duration and intensity. Unilateral ablation of all of areas 3-1-2, 5 and 7, or *bilateral* ablations intensified all symptoms which appeared after the less extensive lesions. In bilateral preparations when the tissue removed from one parietal lobe was greater than that from the other the deficit was greater in the limbs opposite the *larger* ablation.

Fine motor acts were most noticeably affected. The normal hand or foot was always used in preference to the paretic. When the affected digits were used for fine prehension, lack of precision in direction and extent of individual movements was obvious particularly in grooming which requires highly delicate coördination. Accuracy was increased when the animal focussed visual attention on the act. The grosser movements about the cage showed the same type of deficit; the affected extremities assumed bizarre postures and became tangled in perch or bars.

Effect of emotion.—In contrast to changes seen following ablation of either areas 6 or 4, emotion of any sort in the animal with parietal ablation made movement of the paretic limb more sure and accurate. Thus an animal sitting motionless in a cage would show unusual posture until the sight of food or a rage paroxysm sent it into action. Under these stimuli the affected extremities were moved appropriately and gross movements were carried out almost perfectly.

Postural changes.—Immediately after partial or total parietal ablation the contralateral limbs were limp and dangled usually in more extended posture than was normal. Some eversion of hands and feet, and abduction of the extremities was often present. They appeared paralyzed until during some movement one saw perfect flexion, extension, etc. at all joints. At this stage the extremities, if used, appeared weak and tended to "give" beneath the weight of the animal. With improvement in motor function there was diminution in the limpness and a gradual return to bilaterally similar posture. Often sensory loss was demonstrated by the assumption of awkward

positions, a leg might hang through a bar, the fingers of the hand might be doubled under. Attention by the animal or movement often corrected this fault.

Placing and hopping reactions (Bard 1) disappeared immediately following partial or complete parietal ablations in our series of animals. In *partial* lesions, either unilateral or bilateral, hopping and *proprioceptive* placing returned but *tactile* placing continued absent. In partial lesions of the post-central gyrus which excluded area 3, hopping reactions returned, but if area 3 was included in the ablation neither placing nor hopping responses returned during the period of observation. If the entire region, areas 3-1-2, 5 and 7, was extirpated all forms of placing and hopping responses were permanently abolished. With unilateral lesions, crossed placing (*i.e.*, response of paretic extremities to stimuli applied to normal side), could be demonstrated, indicating a sensory, but not a motor defect in this reflex.

The normal response to bringing an object such as the examiner's finger against the sole of a monkey's foot is a quick, apparently involuntary, grasp of that object. This disappears following removal of area 4 and does not return, although the general behavior of the extremities may in every other way approach the normal. This grasp must be in response to tactile stimuli because it appears after the slightest contact with the sole. It is absent after total ablation of areas 3-1-2, 5 and 7. It is present, however, in animals which, following ablation of 3-1-2, or 1-2 have reacquired hopping and proprioceptive placing, but in which tactile placing has remained absent, thus indicating two forms of response to tactile stimuli integrated either in different regions in the parietal cortex or to a different degree, so that one may at times be present, the other absent.

Tendon reflexes.—Immediately after operation tendon reflexes, as shown by biceps and knee jerks were always diminished. The term *pendulous* characterizes the response. At the same time diminished resistance to passive manipulation appeared and continued for some weeks. Eventually resistance again approximated that of the normal side and the knee jerks then were more active, but hypotonia of mild degree remained throughout the duration of the experiments. No abnormal reflexes, *i.e.*, no Babinski, Rossolimo or Hoffmann responses ever appeared. There was never an increase of resistance to passive manipulation, but diminished resistance was present after all ablations.

Atrophy of muscles followed extensive parietal ablations in one instance (Exp. 1), but was not noted in another similar ablation (Exp. 2). The subject of atrophy is one requiring further study.

Bilateral ablations, either partial or complete, merely increased the intensity of all symptoms. Apparently there was permanent loss, both tactile and proprioceptive, if all of areas 3-1-2, 5 and 7 were removed, as all placing and hopping responses were absent in two mangabeys (Expt. 1 and 2) six months after such ablations. These animals then showed noticeable awkwardness in prehension and diminished resistance to passive manipulation.

In another animal six months after bilateral removal of areas 1 and 2, tactile placing was still absent, although proprioceptive placing and hopping had returned. This animal showed no defect in posture or in gait, but fingers were used less well than is normal (P.C. 7).

Deafferentation.—Following deafferentation of a limb a monkey recovers some motor function. It was thought that if this recovery was sufficient a secondary parietal ablation might then alter motor performance. If this occurred it would indicate interruption of motor efferents from the parietal lobe. In the two animals used, however, the recovery following deafferentation was slight and *secondary parietal ablation produced no change in motor behavior whatsoever.*

Effects of ablations of areas 4 and 6.—For purposes of comparison, a brief outline is here included of the motor syndromes which follow ablation of the precentral motor areas (Fulton, 6), as observed in this laboratory in another series of monkeys.

Removal of area 4, the motor area, produces in the monkey immediate "volitional" paralysis of all the muscles of the extremities, most marked distally and least proximally. Tendon reflexes are depressed or abolished as are placing and hopping responses. There is flaccidity at all joints followed in the third week by transient spasticity of the digits (Denny-Brown and Botterell, unpublished). The impairment of fine movements of the digits is permanent. All movements are noticeably less well coordinated when under excitement, rage or any other emotion.

Areas 6 and 4-s. The most obvious effect of removal of area 4-s, the "strip" region of Hines (9) lying between areas 4 and 6, is transient spasticity of the extremity with loss of the finer movements of the digits. The increase in resistance to passive manipulation becomes gradually less, but fine digital prehension is never reacquired. Ablation of area 6 causes reflex grasping with some temporary increase in resistance to passive manipulation and impairment of skilled movements; recovery following unilateral ablation of area 6 is nearly complete.

DISCUSSION

The motor deficits which follow any cortical ablations are thus alike in that they affect most severely the distal portions of the extremities, in particular fine movements of the fingers; and that, in every instance, tendon jerks and resistance to passive manipulation are altered temporarily. But the motor syndrome which follows precentral lesions may be clearly distinguished from that of a postcentral purely by observation of behavior. Analysis of each syndrome brings out the following differences: (i) A true motor paresis follows ablation of either area 4 or 6; movement is inaccurate and there is inability to perform fine movements; (ii) attempted finger movements are slow and remain without individual components even after maximum recovery has occurred. Following parietal ablations fine movements are also inaccurate, but this is corrected by close visual attention; all movements are quick, and the fingers are capable of individual discrete movements although they are poorly adjusted in range and direction. (iii) A noticeable loss of tactile and position sense always accompanies the motor deficit of a parietal lesion. (iv) Emotional disturbances render fine movements less well performed after any motor area extirpation, but better performed after any postcentral lesion. Thus, following a lesion of the motor

cortex either in a spastic or flaccid state emotion always makes motor activity less controlled than if the same act is carried out under quiet conditions without excitement. A spastic limb in particular becomes uncontrollable, forced grasping will become more extreme and all the associated and involuntary movements are brought into play, making voluntary and purposeful accomplishment impossible. These associated movements never appear with parietal lesions.

As in Bard's series of postcentral ablations tactile placing in our animals was more greatly affected by parietal lesions than was either hopping or proprioceptive placing. The placing reaction to tactile stimuli was permanently abolished in all ablations of the postcentral gyrus or of the entire parietal lobe. In one instance, following ablation of areas 5 and 7 only, tactile placing returned after a short absence but the threshold was then higher than before operation. In every case of parietal ablation proprioceptive responses were also altered, but to less degree if the ablated area was incomplete. Immediately following removal of areas 3-1-2, or 5 and 7, these proprioceptive responses were absent. Shortly they reappeared but were less brisk and required greater stimuli. Later they returned to nearly normal level. They were less affected by ablations of areas 5 and 7 than of 3-1-2 or, of 1-2. Following bilateral removal of areas 1-2, 5 and 7 in one instance and of areas 3-1-2, 5 and 7 in another, hopping and proprioceptive placing, as well as tactile placing, were permanently lost.

These findings are in agreement with those of Bard that cortical parietal regions have less influence on the normal response of proprioceptive placing than of tactile placing. Proprioception must, however, be represented cortically in postcentral regions since slight though permanent alteration in response may appear after small parietal lesions and since proprioception is permanently affected (as demonstrated by hopping and placing) if all the parietal lobe is removed from both hemispheres.

The "hypotonus" or decrease in resistance to passive manipulation which was present to some degree in all operated extremities may be also accounted for by proprioceptive loss. If the normal status at a joint either resting or moving is maintained by the reflex lengthening and shortening reactions of muscles connected with that joint then alteration in the status of the stretch reflex will alter the total response of that joint. As the stretch reflex is reduced so resistance to passive manipulation will be less. In the extreme of tabes a flaccid joint results and the "hypotonus" of the parietal syndrome may well be due to disturbance of the same mechanism. Following parietal lesions, however, knee jerks were only temporarily diminished, and later in some case appeared to be increased, so that serious permanent impairment of this reflex arc cannot have occurred.

In one animal the arcuate fibers at the depth of the left central sulcus were cut at a first operation. Transient, true motor paresis followed, perhaps due to pressure on the Betz cell area at the time of operation. Following complete recovery from this, six months later, the left parietal lobe was

excised. Alterations in motor performance following this were of the degree and type seen after primary ablation of this area. The effect on motor performance of such a parietal ablation cannot then be due to a direct effect on motor cortex. The same effects have been observed when a postcentral lesion is made after recovery from complete ablation of area 4.

Localization within the parietal lobe.—In this series there was some indication of functional localization within the parietal lobe, but most evidence pointed to a general and non-specific type of functional activity. Lesions of the postcentral gyrus (areas 3-1-2) caused temporary changes in motor performance, and in response to proprioceptive stimuli. Tactile placing response was permanently lost. Partial lesions of the postcentral gyrus (areas 1-2) were also made leaving areas 3, the posterior lip of the postcentral sulcus, untouched. This was done to be certain that no inadvertent damage to motor area 4 had occurred. The effects on motor performance were similar to those following ablation of areas 3-1-2, except that in the smaller lesion tactile placing was only temporarily impaired.

Bilaterality of function is much less noticeable in the parietal than in the frontal lobe. Ablation, either at a first or at a second operation, was followed by little if any effect upon the ipsilateral extremities; also contralateral changes were more enduring and exhibited less recovery once the initial reorganization had taken place, than did motor functions following precentral lesions.

SUMMARY

1. The alterations in motor performance which follow ablations of the parietal lobe have been compared with those due to ablation of areas 4 or 6. Unilateral removal of any of these three areas produces motor disability which in each instance is characterized by relative disuse of the operated, as compared with the normal extremity, and by the greater involvement of distal joints and movements, as compared to proximal. The motor syndrome produced by ablation of any one of these areas may, however, be clearly distinguished from that produced by either of the other two (see Discussion).

2. As demonstrated by placing and hopping responses, tactile deficit appears following unilateral or partial ablation of either areas 3-1-2, 1-2 or 5 and 7. Proprioception is much less affected by these parietal lesions, but complete bilateral removal of all parietal tissue permanently abolishes the hopping and the tactile and proprioceptive placing reactions.

3. Absent or diminished knee jerks, together with diminished resistance to passive manipulation appear immediately after all parietal ablations. Later knee jerks may become hyperactive, but resistance to passive manipulation never becomes increased.

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SOMATIC AND AUTONOMIC REFLEXES IN SPINAL MONKEYS*

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INTRODUCTION

IT HAS LONG been known that somatic reflexes tend, after spinal transection in primate forms, to be depressed completely for some hours, days or even for weeks; eventually they return, however, and certain reflexes may even become hyperactive. Autonomic reactions, *e.g.*, sweating, vasoconstriction and piloerection, also appear in spinal man. No studies, however, appear to have been made of the time of appearance of these autonomic reflexes in relation to the return of somatic responses in primate forms. Since observations of this character are essential for the elucidation of the theory of levels of autonomic function, the following study was undertaken.

HISTORICAL

Nasse (1839) observed that the temperature of the hind legs of an animal with a severed dorsal cord will rise once more when one destroys the isolated distal portion of the spinal cord. Goltz and Freusberg (1874) noted after destruction of the spinal cord in the dog that the vessels of the lower extremities ultimately recovered their habitual tonus. Similar conclusions were reached by Gergens and Werber (1876) on the frog. Further observations along these lines were contributed by Goltz and Ewald (1896). They felt that the lower portion of the spinal cord was unnecessary for the maintenance of the vascular tone of the hind part of the body, and that vascular tone depended upon local adjustments which could be maintained independently. Sugar and Gerard (1940) sectioned the thoracic spinal cords of rats and found flexion reflexes immediately after the operation, crossed reflexes after 3 days, and scratch reflexes after 11 days. The bladder became automatic in 7 to 10 days. In some of their rats following a month of characteristic spinal reactivity, further sensory and motor recovery began slowly to occur, and the investigators were led to conclude in these instances that true anatomical and physiological regeneration had occurred. Thauer (1935) demonstrated that the rabbit was able to maintain its body temperature in a fairly efficient manner after a period of time following cervical cord section. Popoff (1934) described recovery of temperature regulation following destruction of the spinal cord and cervical section of the vagi in the dog. He concluded that this regulation was made possible by peripheral plexuses and ganglia. Hermann, Morin, and Cier (1937) studied the local effects of cooling the paw of the dog before and after section of the sciatic nerve. The denervated paw was slightly warmer than its homologue during the first 2 or 3 weeks following the section. The denervated limb, however, cooled to the same degree as the one with intact innervation (18–20°C.). These authors (1938) emphasized that recovery of vascular tonus took place approximately 4 weeks after destruction of the distal portion of the spinal cord, but they noted that the degree of local cooling was now somewhat less than before ablation of the cord. After cutting the sciatic nerve on such an animal, and allowing 2–3 weeks for degeneration, they observed that the temperature of the denervated extremity now fell in excess of 20°C. after immersion in the refrigerant mixture. Issekutz, Jr. (1937) reported a recovery of ability to maintain normal body temperature in the dog under ordinary room conditions. The same findings were reported in the

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cat by Issekutz, Leinzinger, and Issekutz, Jr. (1937). These results, however, were not confirmed by observations of Sherrington (1924), Freund (1922), or Clark (1940). The experiments of Bowen, Coombs, and Pike (1922) on cats indicated the functional dependence of the peripheral ganglia of the sympathetic system upon the central system.

Cannon (1930) has been able to show that cats, dogs, and monkeys deprived of their ganglionated trunks from stellate to sacral ganglia on both sides continue to live without apparent difficulty in the sheltered surroundings of the laboratory. Zuckerman and Ruch (1934) reported that the changes in plantar temperatures of macaques which normally followed abrupt fall or rise in environmental temperature, were slower and much less marked after the effects of spinal shock had worn off.

The observations of Head and Riddoch (1917) and Riddoch (1917) on war injuries brought to light many interesting facts concerning immediate and late results of spinal cord injury in man. The immediate effects of complete transection were absence of all reflexes below the lesion, paralysis of the rectum and bladder, and total loss of activity of the sweat glands. Later the reflexes returned and became exaggerated, the rectum and bladder began to function regularly, and the skin became soft and moist. Leriche and Fontaine (1927) reported that low thoracic section of the cord leaves normal vasomotor reactions in man unchanged. They found after extensive destruction of the cord (D8-D10), that all vasomotor reactions persisted in the lower limbs at the end of three months. They concluded that these effects were due to the existence of extramedullary vasomotor centers which one finds partly in the sympathetic nervous system, and partly in the walls of the vessels themselves.

Studies on somatic reflexes after spinal section have been carried out by a number of observers, including Sherrington (1899), Fulton and Sherrington (1932), Fulton and Keller (1932), Fulton and McCouch (1937), and Hinsey and Markee (1938). The return of knee jerk and ankle jerk reflexes, toe signs, withdrawal reflexes, and the like, have been intensively followed and recorded. A number of investigators, including Cooper and Sherrington (1933), Fulton and McCouch (1937), and Hinsey and Markee (1938) have noted the appearance of areflexia in the late stages of spinal section and in monkeys have established the connection between this condition and degeneration of the sciatic nerves (owing to pressure).

Brown-Séquard (1852) credited Edwards with the observation that chilling of a single part of the body, such as the hand or foot, caused a fall in temperature of the other parts of the body without inducing measurable changes in body temperature. Brown-Séquard water produced painful of temperature of non- nges calorimetrically in man. We came to the conclusion that this same immersion test could be applied to monkeys as a convenient means of observing the rate and amount of return of vasomotor function in these animals after spinal transection.

METHODS

Female monkeys (*Macaca mulatta*) were used, their skin temperature being recorded by means of a Leeds-Northrup machine (Micromax), which gave continuous recordings of temperature. The monkey was placed in a holding box without anesthesia. It was restrained in a symmetrical position with both feet free, care being taken that the circulation to the lower extremities was not embarrassed. Waterproofed leads were applied to the palmar surface of the web between the hallux and the first toe on each extremity, and were held in place by a strip of adhesive tape applied loosely around the foot. Simultaneous recordings were made from a lead inserted in the rectum. The experiments were conducted in a small closed room which could be maintained free of drafts at a temperature of 78°F. After a control period, one lower extremity, generally the right, was immersed to the mid-calf in a quart jar containing broken ice cubes, and kept immersed for 20 minutes. The lower extremity, incidentally, was used as a stirring rod to keep the ice water moderately agitated. Care was taken to insulate the jar containing ice-water so that the non-immersed extremity would not come in contact with it. Temperatures of the immersed, as well as the non-immersed portions of the body were thus recorded automatically by the Leeds-Northrup instrument. After several control observations, various operative procedures were carried out on the monkeys and the state of the somatic reflexes, function of the bowels and bladder, piloerection, and the return of the sweat secretion were noted, in ad-

dition to the vasomotor status. The appearance of sweating was observed by smearing a thin layer of vaseline over the sole of the foot and noting the accumulation of droplets of sweat beneath the film. Section of the cord was carried out at various levels, in addition to supplementary procedures, to be mentioned later. The autonomic functions of the animals were studied in some cases immediately after the operation, and in others within a few hours after the operative procedure. As many of the animals were studied from day to day as could be accommodated; in most instances from three to four readings per week were obtained on each animal. The spinal transections produced, in all instances, the well known condition of "shock." Special postoperative attention was paid to care in the attempt to keep the animals alive as long as possible and to avoid pressure on their sciatic nerves. Manual expression of urine from the bladder was carried out until that organ became automatic. The animals were placed on a thick straw bed in their cages; the holding boxes were well padded to avoid pressure on the buttocks. At the conclusion of the series of experiments the animals were sacrificed and at autopsy the level of the operation was identified. The cords were sectioned longitudinally through the site of the operation and stained with hematoxylin and eosin, silver stain for axis cylinders, myelin sheath stain, and toluidin blue stain. The sciatic nerves were removed, in most instances, and stained with osmic acid.

RESULTS OF SPINAL TRANSECTIONS

Skin temperature readings in the normal animal are illustrated by the first monkey in this series. This animal (No. 1141) was an immature female *Macaca mulatta* monkey weighing 2.3 kg. Control studies showed that the skin temperature of the lower extremities seldom remain at a fixed level, even with the animal at rest in a room with constant temperature. Slow rises and falls are the rule. The variations in temperature are not abrupt, but over the course of an hour the fluctuations may amount to a degree or more, Fahrenheit. The average temperature in the web of the hallux and first toe is approximately 90°F. in a room temperature of 78°F. The usual rectal temperature under these conditions is 101°F. Immersion of the right lower extremity in ice water produces an abrupt drop in temperature in the left, or non-immersed extremity (Fig. 1). The average fall in 4 such observations on this animal was 4°F. in 20 min.

EXPERIMENT 1. *Spinal transection in macaque at Th5; 5 months survival; return of crossed cooling on 42nd day. Removal of spinal cord below transection; abolition of crossed vasomotor reflexes; sacrifice 5 days later.* [No. 1141]

First operation: On January 14, 1939, under ether anesthesia, a laminectomy was performed under aseptic conditions and the cord transected at the 5th thoracic level. Examination immediately after the animal had recovered from the effects of the anesthetic revealed completely flaccid lower extremities, all reflexes in the lowers being abolished. There was complete analgesia to pin prick below the fifth dorsal level. The lower extremities were warm and dry, and piloerection was absent. Gradually the somatic reflexes began to make their appearance. A flickering response of the hallux on stimulation of the outer aspect of the sole was obtained 24 hours after the operation. An adductor reflex was present in 48 hours, knee jerk reflex in 96 hours, ankle jerk in 6 days, and flexor withdrawal in 7 days. The reflexes remained very active as long as this animal was kept alive. Fifty-one days after the operation vigorous flexor "spasms" made their appearance. These episodes would occur spontaneously, yet could be induced readily by stimulation of the perineum. The legs would draw up, the toes would spread, and the animal would make slow running movements to the accompaniment of vigorous tail wagging. The bladder and bowels became automatic by the end of the 6th day, sweating and piloerection appeared on the 14th day after the operation. At no time, however, were these two latter functions as active as they were before the operation.

Attempts to cool the left foot by immersion of the right in ice-water immediately and

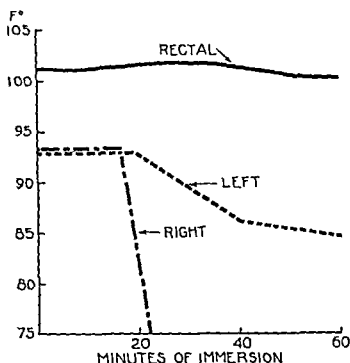


Fig 1 (Expt 1) Left (See text)

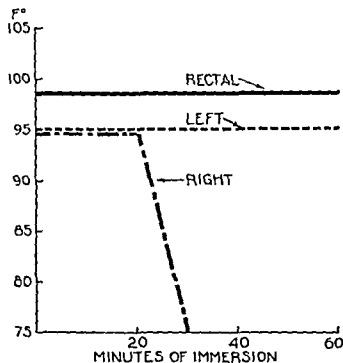


Fig 2 (Expt 2) Right

for a number of days after the operation were unsuccessful. The skin temperature of the lower extremities was relatively high (Fig 2). Specifically, the temperature of the lower extremities was 96°F, while the rectal temperature was now 97.5°F. On the 42nd day after operation, a significant indirect cooling response made its appearance, as indicated in Fig 3. A summary of the rate of appearance of these functions in this, as well as the other animals on which transections of the cord were performed is shown in Table 1.

The leads were now placed so that one was attached to the left foot, one to the right foot, and one to the web between the hallux and index finger of the left upper extremity. The right foot was then immersed in ice water. During this period of immersion the temperature of the left hand dropped 6°F while the temperature of the left lower extremity was dropped 3°F. After the indirect cooling reflex had thus become well established, a tourniquet was applied to the right thigh, inflated to 100 mm Hg, and maintained for 15 min while the right foot and calf were immersed in ice water. The temperature of the

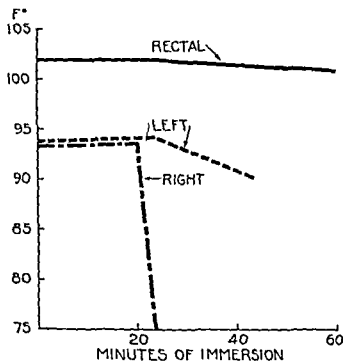


Fig 3 (Expt 1) Left (See text)

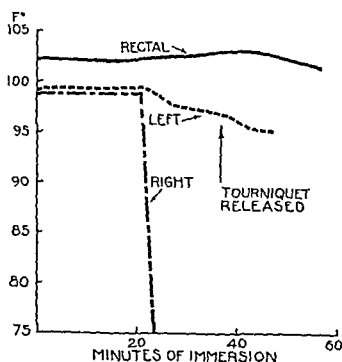


Fig 4 (Expt 1) Right

opposite lower extremity was lowered in spite of the fact that blood was not returning from the immersed extremity. A further fall occurred, however, when the tourniquet was released (Fig. 4).

Second operation—Removal of the distal two-thirds of the spinal cord. On June 23, 1939, under intraperitoneal amytal anesthesia an extensive laminectomy was carried out in order to expose the entire spinal cord below and including the site of the previous operation. It was possible to section the cord one segment above the previous transection and to remove all of the cord distal to that point. Bleeding was controlled with difficulty, but the animal survived the operative procedure. Reflexes, when they were tested 24 hours later, could not be obtained in the completely flaccid lower extremities. The bladder had lost its automaticity and was once more distended. The sensory level was now at D4.

Skin temperature studies showed levels which paralleled and closely approximated the rectal temperature. Once more the indirect cooling response was lost (Fig. 5). However, 0.2 cc. of adrenalin (1-1000) intramuscularly produced a prompt fall in temperature of the left foot after immersion of the right foot in ice-water had failed to do so (Fig. 6). The animal was sacrificed 5 days after the second operative procedure.

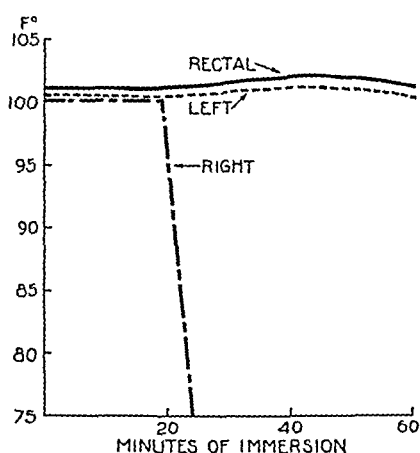


FIG. 5 (Expt. 1). Left (See text).

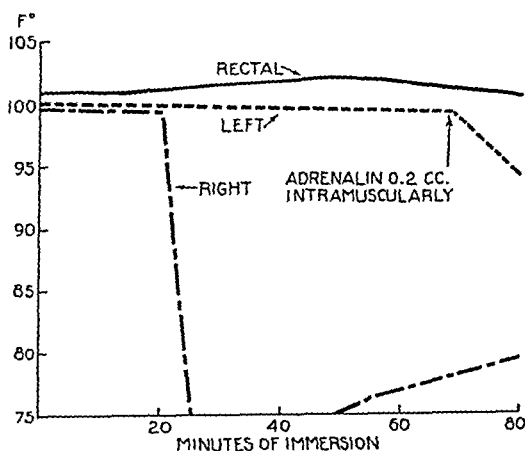


FIG. 6 (Expt. 1). Right.

Microscopic examination of the cord at the site of the first operative procedure showed that the cord section had been complete. The sciatic nerves, removed at the time of autopsy, were found to be intact except for early Marchi degeneration.

Eight other monkeys were used in this series; the results are recorded in Table 1.

The third monkey (No. 1177) in this series showed an average drop of 3°F. in the non-immersed lower extremity before spinal section. As part of the preoperative observations, the effect of a tourniquet applied to the immersed extremity was noted. In the non-immersed foot there was a rather prompt fall of 2°F. in temperature; the curve levelled off, with a further drop when the tourniquet was released.

Spinal transection. Macaque no. 1177. Operation. On February 4, 1939, the spinal cord was transected at the 12th dorsal level. Examination of the animal after it had fully come out of the anesthetic showed loss of sensation below the groins and loss of motor power in the lower extremities, as well as paralysis of the bladder and bowels. A slight degree of piloerection was observed one hour after the operation, and sweating was present during the first day in the lower limbs. The bladder and bowels became automatic by the 3rd day,

Table 1—Appearance of somatic and autonomic reflexes after spinal transection in monkeys
(Time intervals in days unless otherwise specified)

Monkey No	1141	1156	1177	1194	1187	1186	1201	1211	1224
Average drop of temperature of non immersed foot preoperatively (°F)	4	5	3	3 5	3	3	4	2 5	4
Level of transection	5D	7D	12D	6D	10D	1D	10D	1D	6C
Return of function below level of section (days postoperative)									
Flickering response of hallux	1	1	*	4	4 hr	2	1	3 hr	1
Adductor	2	20 min	*	1 hr	2	1	1	3 hr	1
Knee jerk	4	5	*	1 hr	2	1	1	3 hr	1
Ankle jerk	6	7	*	1	5	1	1	3 hr	1
Flexor withdrawal	7	7	*	19	9	22	*	8	*
Automatic bladder	6	5	2	7	5	16	11	11	5
Automatic bowel	6	5	3	3	5	4	8	3	5
Sweating	14	12	1	14	5	22	11	17	*
Piloerection	14	5	1 hr	4	4	22	11	17	*
Crossed cooling response	42	26	16	52	17	39	*	*	*
Average fall in temperature of non immersed limb (°F)	3	3 5	3	3	2	2 5	*	*	*
Sacrifice or subsequent operation, days after 1st operation	160	145	141	128	100	117	14	19 (pneumonia)	10 (pneumonia)

* Did not appear

and the indirect cooling response returned on the 16th day. No evidence of return of somatic reflexes was ever found in the lower extremities. It is significant that the indirect cooling response was maintained from the 16th day throughout the remaining period of observation, totaling 141 days after the operation. At time of autopsy and in the subsequent microscopic sections the cord was found to have been severed completely at the 12th dorsal level, and the entire lumbar cord to have been softened and replaced with glial and connective tissue. Specimens of the sciatic nerves stained with osmic acid showed evidence of abundant Marchi degeneration.

The fourth monkey (No. 1194) showed an average preoperative drop of 3.5°F in the non-immersed lower extremity.

Macaque no. 1194 Spinal transection. On February 18, 1939, a transection of the cord was performed at the 6th thoracic level. Somatic reflexes were well established within the first week. The bladder became automatic on the 7th day, piloerection was observed on the 4th day, sweating on the 14th day, and the indirect cooling response made its appearance on the 52nd day. These reactions were maintained until the animal was sacrificed 128 days after the operation. Shortly before exitus the effect of adrenalin, 0.25 cc of 1-1000 solution intramuscularly, was tried. A prompt drop of 6°F in the lower limbs during the ensuing 20 min. occurred. This fall was twice as great as that produced by immersion of the opposite lower extremity of this animal.

OTHER LESIONS—CONTROL OBSERVATIONS

Monkey No. 1196, belonging to species *Cercopithecus mona*, was brought into the laboratory by its owner who stated that the animal had been af-

flicted with progressive paralysis of its lower extremities for 2 weeks. At the time of examination on February 24, 1939, the animal was unable to move its lower limbs, deep reflexes of the lower extremities were hyperactive, and there was hypesthesia below the level of the umbilicus. A drop of 4°F. was found in the non-immersed lower extremity. Autopsy revealed compression myelopathy at the 7th dorsal level as a result of tuberculous granulation tissue in the epidural space.

Monkey No. 1203 proved to have an average preoperative drop in the non-immersed extremity of 4.5°F. The antero-lateral portion of the cord on the left was destroyed by operation at the 5th dorsal level. Five hours after the operation, when the animal was well out of the anesthetic, there was hypotonia of the left leg, which was moved slightly, and pain sensation was reduced on the right lower extremity. Upon cooling the right foot in ice-water 20 min., the temperature of the left fell 2°F. Two days after the operation a drop of 4°F. was obtained; 10 days after the operation there was a drop of 3.5°F. Sixteen days after the first operation the wound was opened, the cord again exposed and a transection made immediately above the previous lesion. The course after the 2nd operation followed that of other spinal transections. The animal went through a period of spinal shock, somatic reflexes began to make their appearance within 24 hours. An appreciable indirect cooling reaction made its appearance on the 27th day after the second procedure, *i.e.*, a drop of 3°F.

Monkey No. 1222 had a preoperative drop in temperature of 5.5°F. in 20 min. in the non-immersed lower extremity. On April 20, 1939, a right lateral semisection was performed at the 9th dorsal level. Examination 4 hours after the operation showed a completely flaccid right lower extremity, with preserved function on the left. Sweating and piloerection were absent on the right and present on the left. Sensibility to painful stimuli was absent on the left lower extremity. The skin temperature of the right foot was 97° and the left 88°F. Immersion of the left foot in ice-water produced a drop of 3°F. in the non-immersed right leg. Sweating and piloerection were much in evidence in the right leg 5 days after the operation, at which time the indirect cooling drop amounted to 5°F. Forty-one days after the operation a drop of 6°F. was obtained. On June 27, 1939, or 68 days after the initial operation, transection of the cord was performed at the 5th dorsal level. After the animal had blown off the ether it was found to have lost all voluntary motion in the lower extremities. The deep reflexes were present and hyperactive on the right but were absent on the left lower. Cooling the left leg in ice water did not produce a drop in temperature in the right, yet there was a drop of 3°F. in the right foot after the injection of 0.2 cc. of adrenalin (1-1000) intramuscularly.

Animal No. 1225 showed preoperative drop in temperature of the non-immersed lower extremity amounting to an average of 5°F. On May 26, 1939, through a transperitoneal approach, a bilateral lumbar ganglionectomy was performed, the ganglia on each side being removed from the level of the

renal vein down to the point where the trunk disappeared behind the common iliac vessels. Three days after the operation the skin of the lower extremities was warm and dry. The temperature of the lower extremities was recorded as 100°F. in each foot, the rectal temperature being 102°F. The indirect cooling reaction could not be produced. This animal was not observed again until June 15, 1939, when the temperature in the non-immersed lower extremity dropped 4.5°F. On June 19, 1939, the femoral and sciatic nerves on the right were sectioned. The animal now had a completely flaccid right leg, the skin temperature of which was cooler than the left. Immersion of the right leg in ice-water produced a drop of only 2°F. in the left. Repetition of this same experiment with a tourniquet applied to the right leg so as to occlude the flow of blood from that extremity gave negative results in so far as the temperature of the left leg was concerned. On June 22, 1939, with the animal in the holding box and leads attached to both lower extremities, an intramuscular injection of 0.3 cc. of adrenalin (1-1000) reduced the temperature of the right foot from 91.5 to 87°F. in 20 minutes, and the left from 100 to 96.5°F. in the same period of time. On June 24, 1939, a transection was performed at the 5th dorsal level. On the following day the knee jerk and ankle jerk were present but hypoactive in the left leg. Now the skin temperature of the left lower extremity paralleled and closely approached the rectal temperature but the temperature of the right lower extremity remained from 2 to 4°F. below the figure for the left. Immersion of the right lower extremity in ice-water produced no drop in the temperature of the left which, however, showed an immediate drop upon the injection of adrenalin intramuscularly.

Monkey No. 1179 showed an average preoperative temperature drop amounting to 4°F. in 20 minutes. On May 31, 1939 a bilateral lumbar sympathetic ganglionectomy was performed in a manner similar to that described for Monkey No. 1225. On June 1, 1939, the skin of the lower limbs was warm and dry, in contrast to that of the upper which was cool and moist. The temperature of the skin of each lower extremity was 100°F., the rectal temperature 102°F. Cooling studies on June 2, 1939, showed a negligible drop in temperature of the non-immersed left lower extremity. The next time the animal was examined, on June 16, 1939, the lower extremities, although dry, were much cooler to the touch than they had been immediately after the operation. The indirect cooling response had, by now, returned. On June 20, 1939, the spinal cord was sectioned at the 11th dorsal level. On the following day the skin temperature recordings of the lower extremities were the same as they were prior to the operation on the spinal cord, with indirect cooling of 1.5°F. On June 22, 1939, there was an indirect cooling response of 2°F. When a tourniquet was applied to the right leg, however, the drop was only 1°F. On June 26, 1939, the indirect cooling response amounted to 3°F. The fall in temperature was prompt in both lower extremities after the injection of adrenalin 0.3 cc. intramuscularly.

CONCLUSIONS

1. Immersion of one foot of a monkey in ice-water will cause a drop in temperature of 4–5°F. in the opposite lower extremity, as measured by the Micromax recorder. Since this response returns some time after spinal transection, it is unlikely that painful stimuli play the chief rôle in its production. This indirect cooling reaction is present, though less marked, in the normal monkey, if the immersed lower extremity is first constricted with a tourniquet. Release of the tourniquet is followed by a further drop in the skin temperature of the opposite extremity. These observations would indicate that the indirect cooling reaction is mediated both by the nervous system and the blood stream.

2. The term "shock" applies equally to autonomic and to somatic reactions. The immediate effect of transection of the spinal cord of monkeys from the 6th cervical to the 12th thoracic levels is the well recognized state of hyporeflexia, characterized by abolition of all somatic reflexes, paralysis of bowel and bladder function, dryness of the skin, absence of piloerection, and vasomotor paralysis, the latter condition being evidenced by skin temperatures in the feet of a value which parallels and closely approximates the rectal temperature. During this period of "shock" cooling one lower extremity does not cool the opposite limb.

3. The return of somatic reflexes precedes the return of autonomic functions. Certain somatic reflexes may appear within one hour after the spinal transection, and many somatic reflexes become fully established within one week after the operation. Automaticity of the bladder and bowel usually makes its appearance shortly after the somatic spinal reflexes are established. Sweating and piloerection appear usually one week after the bowels and bladder become automatic. Sweating and piloerection, however, are less efficient than before operation. The indirect cooling response, deficient during the period of "shock," makes its appearance after several weeks. In general, the higher the level of the transection, the longer period of time required for this response to return. Upon its establishment following operation, the indirect cooling response is less well developed than before spinal section. Removal of the portion of the cord distal to the original section will once more abolish the indirect cooling response. Injection of adrenalin, however, still causes a prompt fall in temperature in the non-immersed foot when cooling the opposite lower extremity fails to do so.

4. A slowly progressive tuberculous process resulting in a practically complete transverse lesion at the 7th dorsal level led to no significant depression of the crossed cooling response when this reaction was tested two weeks after the onset of the signs of paraplegia.

5. Partial lesions of the cord affect the crossed cooling response much less than complete transection. Lateral semisection of the cord at the 9th dorsal level depresses, but does not abolish this response. Subsequent transection of the cord above the level of the semisection abolishes the crossed

cooling response, in spite of the fact that adrenalin will result in a drop in temperature of the lower extremities.

6. Lumbar (preganglionic) sympathectomy obliterates the crossed cooling response which returns some time within 3 weeks; the stimulus for the response can be elicited through a denervated lower extremity, but not when this denervated extremity has its return blood supply cut off by a tourniquet. Spinal section at the 11th dorsal level now depresses, but does not obliterate the indirect cooling response.

7. Adequate nursing care is imperative in the management of spinal monkeys, and special precautions must be taken to prevent pressure atrophy of the sciatic nerves.

SUMMARY

Spinal cord lesions were made in 14 monkeys. In 9 animals transections were performed at various levels, and observations made relative to the rate and extent of return of somatic and autonomic functions below the level of the lesions. In 3 monkeys the effect of partial lesions of the spinal cord was noted. In 2 such animals observations were made relative to the effect of transverse cord lesions after bilateral lumbar sympathectomy. The term "shock" applies equally well to the autonomic as to the somatic systems following complete lesions of the cord. The return of somatic reflexes precedes the reestablishment of such autonomic functions as piloerection, sweating, and vasoconstriction. Although the autonomic functions return after spinal transection, they do not reach the same degree of efficiency that they exhibit in the normal animal. Cooling of one lower extremity in ice-water normally produces a drop in temperature in the non-immersed lower extremity, but fails to do so during the period of "shock" following spinal transection, or for a short period of time following lumbar (preganglionic) sympathectomy. This indirect cooling response is temporarily depressed after partial lesions of the cord.

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NERVE ACTIVITY ACCOMPANYING FASCICULATION PRODUCED BY PROSTIGMIN

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THE OCCURRENCE of fascicular twitching of muscle following the administration of prostigmin or eserine has been recognized for many years^{1,2}. Although it has been thought to result from the action of these drugs on the muscle or the motor end plate, there are certain features of the response which are difficult to explain on this basis.

Thus it is important to observe that the muscle contraction produced by eserine is a grossly visible twitch. It is the movement which results from the simultaneous contraction of a number of muscle fibers. That this group of muscle fibers acts in a synchronized fashion suggests that the contracting fibers may all be members of the same motor unit, and that they are all responding together to a nerve impulse initiated by eserine. In order to examine this possibility, Langley and Kato³ determined the effect of nerve degeneration on the fasciculation produced by eserine. Section of a nerve causes no immediate change in this fasciculation in the paralyzed muscle, showing that the twitching is not of spinal cord origin. However, when the nerve has been cut and allowed to degenerate, fasciculation no longer is seen in the denervated muscle following the administration of eserine. These facts strongly suggest that the effect of eserine may be to stimulate nerve activity, and to produce muscle twitching indirectly rather than by acting upon the muscle itself.

On the other hand, Eccles¹ in reviewing this problem, points out that eserine has little effect upon isolated nerve. The twitches produced by eserine are prevented by the administration of doses of curare much smaller than those required to block neuro-muscular conduction. If the impulses were originating in the nerve, one might expect that they would be stopped by curare only when neuro-muscular block was established. Eccles concludes from this that the effect both of curare and of eserine is at the myo-neural junction.

In view of the incompatibility of these two opinions, it was felt advisable to search for evidence of motor nerve excitation by eserine in the intact animal. If eserine does stimulate the motor nerve, the impulse that proceeds distally to produce the fascicular twitch must also travel antidromically along the motor nerve and in the anterior root. Here, separated from sensory activity, these antidromic impulses should be demonstrable. It was to examine this possibility that the following experiments were carried out.

The experiments were performed on cats under chloralose anesthesia, this drug having been selected because it is considered to have little effect

* Supported by the Kirby McCarthy Funds

upon neuro-muscular conduction⁵. Jugular and tracheal cannulae were inserted under ether anesthesia, after which the chloralose was given intravenously in doses sufficient to keep the animals quiet without reducing reflex excitability. The usual dose was 150 mg. for a 2-3 kg. cat. A lumbar laminectomy was then performed, and the lower lumbar and sacral roots

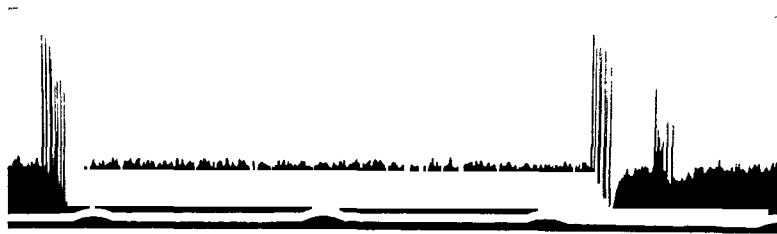


FIG. 1. Action potentials recorded from an anterior root of the cat following the administration of prostigmin, 0.25 mg. Time 0.3 sec.

were exposed within the spinal canal. The sensory roots were divided at a point near their entrance into the cord. The 5th lumbar and 1st sacral motor roots were isolated and sectioned near the cord in order to provide a long intra-spinal segment. With such a preparation it was possible to record from the anterior root, and to observe any impulses coming to it from the peripheral nerve. Action potentials were recorded by means of ringer-agar brush electrodes and a condenser-coupled amplifier.

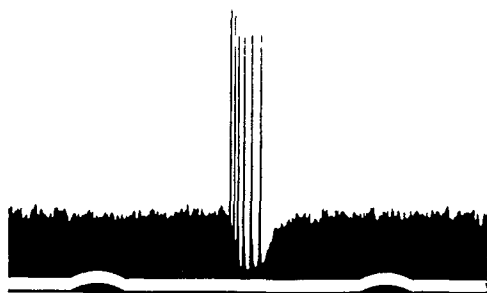


FIG. 2. Single burst of nerve impulses recorded from the anterior root of the cat following the administration of prostigmin, 0.25 mg.

impulses, at a frequency of about 200 per sec. (Fig. 1 and 2). There is a close similarity between the activity recorded from the nerve and that which may be recorded from the muscles under similar circumstances (Fig. 3).

This similarity is also observed in another type of experiment. Following

With this preparation, no activity is normally observed in the anterior root. An occasional nerve exhibited spontaneous activity as a result of the trauma of the dissection, but rarely did this last for more than a few minutes. Following the administration of prostigmin, however, the nerve rapidly becomes active. Coincident with the appearance of fascicular twitching in the muscles (usually after intravenous doses of 0.2 to 0.7 mg. of prostigmin), there are observed rapid bursts of nerve impulses in the anterior root. The individual bursts may contain as many as twenty im-

the administration of prostigmin, it has been observed⁶ that stimulation of a peripheral nerve causes a tetanic contraction of muscle rather than the usual twitch. It was therefore interesting to determine whether this repetitive response of muscle might also be associated with nerve activity. In order to do this, the sciatic nerve was freed in the thigh, and was sufficiently



FIG. 3. Action potentials recorded from the gastrocnemius muscle of the cat following the administration of prostigmin, 0.25 mg. Record obtained with concentric needle electrode during active muscle fasciculation. Time 0.2 sec.

cleared to permit the application of silver electrodes for stimulation. Stimulation of the nerve in this way produces a strong contraction of the calf muscles, and at the same time a nerve volley is conducted up the nerve and may be recorded from the electrodes on the anterior root. After the administration of a small dose of prostigmin, this nerve volley recorded from the anterior root is followed by a short burst of random nerve impulses (Fig. 4). At the same time there is observed the well known repetitive response of the muscle. Following a short, high frequency tetanus, there is again a shower of nerve impulses in the anterior root, which under these circum-

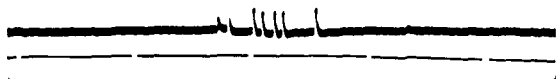


FIG. 4. Action potentials recorded from a few fibers of the anterior root of the cat during stimulation of the sciatic nerve after the administration of prostigmin, 0.25 mg. Time 0.05 sec. The small shock artifact is followed by the conducted impulse, after which there is repetitive activity in one nerve fiber.

stances may last more than a second. If one divides the sciatic nerve distal to the point of stimulation, or injects cocaine into it at this point, the directly conducted impulse remains unchanged, but the nerve activity after the volley is no longer observed. As one would expect, the muscle response is also abolished. It thus appears unlikely that this after-discharge could be the result of any local effect at the site of stimulation, and we must conclude that the repetitive stimulation of the nerve is occurring at a point somewhere near its distal end.

In order to prove that none of the nerve impulses were originating in the proximal portion of the nerve, an attempt was made to record impulses traveling down the nerve after the administration of prostigmin. To do this all the motor roots of the lumbo-sacral region were severed from the cord so that no normal motor activity would occur in the peripheral nerve. A small branch of the peroneal nerve, uncut, was then cleared, and recording electrodes were applied to it in the popliteal space. Under these conditions, one records a constant stream of sensory impulses traveling up the nerve. If, after the intravenous injection of prostigmin, the nerve is cut at a point distal to the recording electrodes, it is no longer possible to find any evidence of activity reaching these electrodes. One must conclude that the segment of motor and sensory nerve lying between the cut root within the spinal canal and the recording electrode in the popliteal space is not stimulated by prostigmin. The antidromic activity recorded from the anterior root must all originate in the terminal portion of the nerve.

If it is actually a portion of the nerve which is stimulated by prostigmin, one might expect that curare, which is thought to have its effect largely upon the myo-neural junction, might block the muscle response without affecting the nerve activity. This does not prove to be the case. In an animal showing active fasciculation as the result of prostigmin, the administration of curare results in prompt cessation not only of the muscle movement, but also of the nerve impulses in the anterior root. This occurs with small doses of curare insufficient to abolish indirect excitability of the muscle. In no instance did curare stop the visible twitching without also stopping the nerve activity, and in no instance did it stop the nerve activity without also stopping the muscle movement. As pointed out by Eccles⁴, the rapidity with which the prostigmin effect is abolished by small doses of curare certainly suggests that the site of action of these two substances is the same.

The close association of nerve activity with movement suggested the possibility that the nerve activity might be the result of mechanical stimulation of the nerve endings by the muscle movement. Another possibility was that there are a few sensory fibers in the anterior root which carry impulses initiated by muscle movement. It was found, however, that stretching or pinching of the muscle did not result in any activity in the anterior root. In order further to examine this possibility, a slightly different preparation was used. A single motor root may be split longitudinally within the spinal canal in such a fashion as to produce two divisions of the root. These two divisions supply overlapping areas within the muscles which they innervate, and it is probable that the nerve fibers, intermingling distally, supply adjacent muscle fibers in many locations. It is thus possible to stimulate one division, and to record from another whose nerve fibers terminate within the muscle thus caused to contract. Under these conditions, following the administration of prostigmin, stimulation of one division does not produce any immediate "after-discharge" in the other. Following long continued stimulation of one division, there was observed on several occasions an in-

crease in the frequency of the bursts caused by prostigmin in the other. This, however, is a slowly developing phenomenon, and appears to differ from the immediate burst of activity which is observed when one stimulates and records from the same root. From these experiments it seems certain that muscle movement itself does not produce the nerve impulses recorded in the anterior root.

Results similar to those obtained with prostigmin were observed following the intra-arterial injection of acetylcholine. This injection was performed in the cat by the introduction of a small cannula into a branch of the popliteal artery. The solution to be tested was injected back through this branch into the popliteal artery. The muscle contraction which is observed following the injection by this technique of 0.1 mgm. of acetylcholine in 1.0 cc. of Ringer's solution is slow and irregular, and is associated with grossly visible fascicular movements of the muscle which persist for several seconds. Coincident with the muscle contraction, there appears a shower of antidromic impulses in the motor root supplying the area. The discharge differs slightly from that associated with the twitchings produced by prostigmin in that there is little tendency for the impulses to appear in bursts. Following the administration of curare (10 mgm.) it was found impossible to obtain either the muscle contraction or the nerve impulses by the injection of acetylcholine.

In this respect acetylcholine and prostigmin differ from certain other substances which are nerve stimulants. Although the intravenous injection of veratrine, guanidine, or tetraethyl ammonium chloride is followed by activity in the motor nerve, which may be recorded from the anterior root of the preparation described, this activity is continuous, does not occur in bursts, and is much more intense than that caused by prostigmin. In addition, it is unaffected by the administration of curare in amounts sufficient to stop the activity reflected in the muscles.

DISCUSSION

The experiments cited above show that the appearance of fasciculation following the administration of prostigmin is associated with the occurrence of antidromic impulses in the anterior root, and that these impulses originate only in the region of the terminal portion of the nerve. The activity of the nerve is not the result of mechanical stimulation by muscle movement or contraction. It has also been demonstrated that acetylcholine is capable of producing similar nerve activity. Since prostigmin is known to lead to an accumulation of acetylcholine in muscle tissue^{7,8}, it is suggested that the stimulation observed following the administration of prostigmin may actually be due to the accumulation of acetylcholine which it produces.

In attempting to determine the site of action of the stimulating substance, several difficulties arise. If one assumes that the impulses are arising in the nerve at its ending, one is immediately faced with the fact that they cease following the administration of curare. This would mean that the ac-

tion of curare cannot be limited to muscle, but that it must also have an effect upon the nerve. If there is actually nerve stimulation by acetylcholine, it must be distinguished from the nerve excitation produced by guanidine, veratrine and tetraethyl ammonium chloride, which is unchanged following the administration of curare in doses much larger than those which completely stop any activity produced by prostigmin or acetylcholine. In other words, the action of curare and of acetylcholine on nerve is a peculiarly specific one, and is probably evidenced only at the nerve ending.

Furthermore, the action of acetylcholine is probably not limited to the nerve, for it has been shown to stimulate denervated muscle⁹, possibly through an effect upon the motor end plate. Thus Harvey¹⁰ observed that quinine prevents the stimulation of muscle by acetylcholine, but does not alter its stimulation by KCl. He suggests that KCl acts directly upon the muscle, whereas the acetylcholine might act upon the motor end plate. If this be its only site of action, one must assume that the impulse initiated by acetylcholine in the motor end plate is conducted not only to the muscle, but also antidromically into the motor nerve, and thus to the anterior root. In order to do this, the impulse would have to pass from end plate to nerve ending, and in view of the many experiments on neuro-muscular transmission which have led to the conclusion that conduction in this location is mediated by chemical agents, such a possibility seems extremely unlikely. It is much more likely that in the same way that acetylcholine stimulates the end plate, it also stimulates the motor nerve ending at the end plate.

In view of the above considerations, we are led to the conclusion that acetylcholine is capable of stimulating both muscle and nerve. This effect may be observed both when the drug is administered intra-arterially, or when it is caused to accumulate in the tissues by the administration of prostigmin. It is possible that the two effects are merely manifestations of the same action of the drug on similarly irritable structures. Whatever the details of this action may be, it would appear that the limited concept of a specific local effect of acetylcholine on muscle at the myo-neural junction is insufficient to explain the observation reported.

SUMMARY

The fascicular twitching observed in animals following the administration of prostigmin is associated with antidromic nerve impulses which may be recorded from the anterior (motor) roots.

Both the fascicular twitching and the nerve impulses are abolished by small doses of curare.

Similar impulses are observed when a muscle is caused to contract by the intra-arterial injection of acetylcholine.

It is suggested that these experiments provide evidence that acetylcholine has a stimulatory effect not only on striated muscle, but on the terminal portions of the motor nerve as well.

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EFFECTS ON RESPIRATION, BLOOD PRESSURE AND GASTRIC MOTILITY OF STIMULATION OF ORBITAL SURFACE OF FRONTAL LOBE

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POSSIBLE autonomic functions of the orbital surface of the frontal lobe have been subjected to very little study. Spencer (1894) exposed this area in the cat, dog, rabbit and monkey, and found in all that slowing or arrest of respiration occurred most readily on stimulation of the "outer side of the olfactory tract just in front of the junction of the tract with the uncinatus." In a larger area lateral, anterior and posterior to this the responses were similar but had a higher threshold. The nine tracings Spencer published to show stimulation in the cat and dog, all had as well a rise in blood pressure. He states that he found no circulatory changes from stimulation of this area in the monkey and his published tracings are in agreement therewith. W. K. Smith (1938) has found an area in the cat's gyrus orbitalis (gyrus compositus anterior) whose stimulation results in respiratory inhibition and a rise in blood pressure. He did not study the orbital surface of his monkeys. Recently Bailey and Bremer (1938) found that stimulation of the central end of the vagus nerve increases the electrical potentials of the orbital surface of the frontal lobe, and the present investigation follows up that finding.

METHOD

Adult domestic cats and monkeys (*Macaca mulatta*) were used in our experiments. The cats were anesthetized with 30-40 mg. per kg. of nembutal, the monkeys with 20-26 mg. per kg., followed by ether for operative exposure, if necessary. Using aseptic technique the orbital surface of the right frontal lobe was exposed by removing the contents of the orbit, its bony roof and the walls of the frontal sinus. The area on the orbital surface of the brain whose excitation produced respiratory arrest was then identified by stimulation with a bipolar copper electrode with the two poles 1 mm. apart and that area was next removed by subpial dissection. The wounds were closed by interrupted suture of anatomical layers. On the day the animal was to be sacrificed for histologic studies of the brain* it was anesthetized with ether, a tracheal cannula inserted, the left orbital surface exposed under light ether narcosis. In a few animals the tracings were made during stimulation of the right orbital surface. Respirations were recorded by means of a sphygmomanometer cuff around the thorax and abdomen attached to a tambour writing on a kymograph. With this apparatus the upstroke represents inspiration. The blood pressure in the common iliac artery (cannulated extraperitoneally) was recorded by a mercury manometer, and gastric movements were recorded by a water manometer connected to a stomach balloon inflated with 50-100 cc. of air. The stimulus was a 60-cycle sine wave alternating current obtained from the house lighting circuit and reduced to appropriate voltage (1-6 V).

RESULTS

From the portions of the gyrus orbitalis of cat and monkey indicated on the diagrams there were obtained: (i) slowing or arrest of respiration; (ii) a rise in blood pressure and (iii) a relaxation of the stomach.

* These studies will be communicated in a subsequent publication.

Respiration. Of these responses the respiratory inhibition had the lowest threshold, frequently appearing with a stimulus of 1 V in both cat and monkey. The features of the inhibition in cats described by Smith were in general corroborated by us. With a stimulus strong enough to stop the respiration this was the commonest sequence: a latent period of less than one second, cessation in full expiration for 5-10 sec., escape with slow shallow respirations, a deep breath after the stimulus ended, resumption of

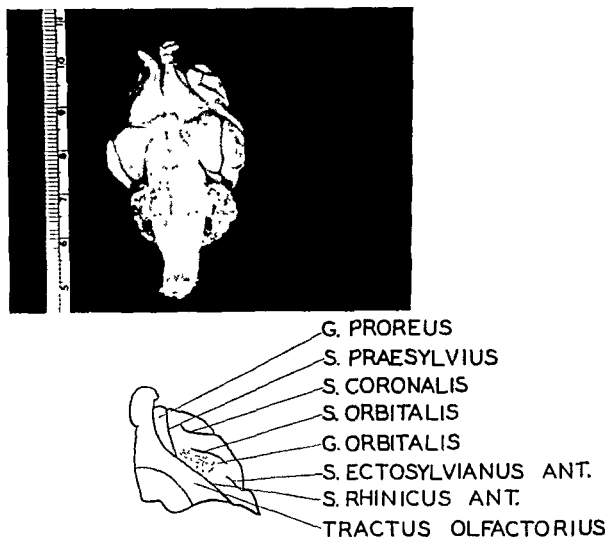


FIG. 1. Cat's brain—basal surface. Diagram below photograph shows orbital surface of left frontal lobe ($\times 2$). From the stippled area in gyrus orbitalis the described effects were obtained.

normal rate and amplitude at once. In the cats interruption also occurred and was maintained at any other phase of the respiratory cycle and some cats inspired slowly throughout the time of the stimulus. A few tracings show several nearly normal breaths after application of the stimulus before arrest appeared. Rarely escape from inhibition was shown by one deep breath followed by arrest again, or by a group of slow deep breaths continuing till the end of stimulation. Frequently there was no deep breath at the end of the stimulus. Rarely following stimulation a number of deep breaths intervened before the normal rhythm recurred; almost never did an

after-discharge of inhibitory effect succeed removal of the stimulus. When the voltage was inadequate to produce arrest the following varieties of inhibitory effect were seen: with decreased respiratory rate there might be either supernormal, normal or decreased amplitude; with unchanged respiratory rate the amplitude might decrease. Various combinations of the foregoing might occur during the application of a single stimulus. The maximum arrest obtained lasted 24 sec. following the application of 6 V. In one animal no respiratory inhibition could be produced.

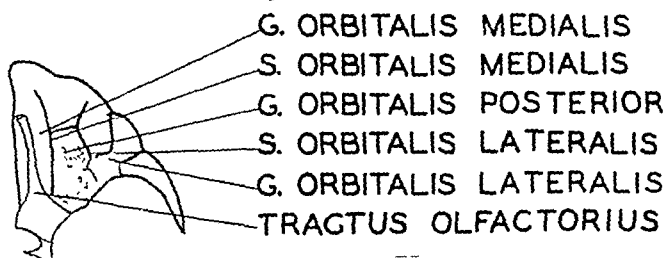


FIG. 2. Monkey's brain—basal surface. Diagram of orbital surface of left frontal lobe is natural size. Described effects were obtained from stippled area in gyrus orbitalis posterior.

Responses from the gyrus orbitalis posterior of the monkey corresponded to these in the cat except that: arrest never occurred in any phase but full expiration; there was frequently a shallow breath before full arrest; a deep breath at the end of stimulation was present more often; and respiratory inhibition occurred in each of the 6 monkeys stimulated.

Any inhibition obtained from the orbital surface outside the stippled area on the diagrams had a much higher threshold and did not occur regularly. In particular we found no inhibition following stimulation of the olfactory tract. Since the boundaries of the area shown depend for their

orientation upon the sulci, which vary from animal to animal, our diagram represents an approximation with a probable error of at least ± 1 mm. at any point on that boundary.

Blood pressure. The changes in blood pressure in the cats were typically as follows: 2-7 sec. after application of the stimulus there was a gradual rise in blood pressure, which continued until the end of a 10-25 sec. stimulus, and was succeeded by a more rapid fall to the original level. The increases were nearly always less than 10 mm., and there was rarely an unequivocal rise in blood pressure unless the stimulus were sufficiently strong to halt the respiration. Three of 10 cats gave no rises in blood pressure at all, although respiratory arrest was obtained in them. In the other 7 cats about 20 per cent of the stimuli producing respiratory standstill caused no certain change in the blood pressure. Since all of the animals in this series had pressures below 70 mm. Hg by the time the extensive operative preparation was completed a part of the failure of response may have been due to shock. The responsiveness of the cortex was usually greater an hour after the exposure than immediately thereafter. Variations in the details of the response were numerous. At times the stimulus was followed by a drop of 2-3 mm. in pressure before the rise occurred; at times the rise began with a latent period of less than 1 sec. or greater than 7 sec.; occasionally during the recovery phase there was a dip below the original level before it was resumed.

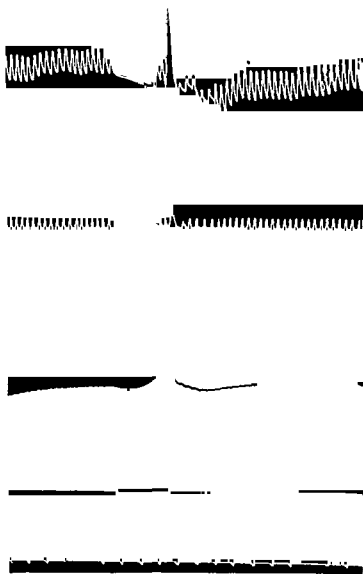


FIG. 3. Monkey No. 3. Top tracing—intra-gastric pressure; second tracing—respiration; third tracing—pressure in common iliac artery; fourth tracing—signal marker for stimulus; fifth tracing—time at 5 sec. intervals.

Stimulus 6 V applied to stippled area shown in Fig. 2. Respiratory excursions superimposed on the level of gastric tonus in first tracing. Maximum gastric relaxation 15 sec. after stimulus off with gradual return toward the pre-stimulation level. After onset of stimulus one respiration at half normal amplitude before the complete arrest. Slight drop and then a rise in blood pressure.

In the monkey the rises in blood pressure were obtained more consistently, were higher, and were often present when the respiration was merely

slowed. In each of the monkeys in which respiratory arrest was obtained, elevations of blood pressure of 10–20 mm. occurred at least once during stimulation and in one macaque the rise averaged 14 mm. for all stimuli arresting respiration. The average detailed behavior of the changes in arterial pressure corresponded to those in the cats and this average was subject to the same variations. In areas outside the stippled region shown on the diagrams rises in blood pressure of more than 2–3 mm. were not obtained from the orbital surface of the frontal lobe in either cat or monkey.

Tonus of gastric muscle. In the state of light narcosis in which the animals were held during stimulation, the gastric wall containing an inflated balloon either maintained a relatively constant state of tonus or exhibited rhythmic fluctuations of contraction and relaxation. Sudden marked spontaneous alterations of tonus were not observed. Sharp relaxations took place, however, upon stimulation.

In the cats such relaxation occurred in only 20 per cent of the stimulations of the appropriate area, and in 3 of 10 cats no relaxation of the stomach took place following any stimulation. In monkeys, however, the responses were clearcut and consistent. Typically there occurred within one second of the onset of the stimulus a brisk diminution of tonus in the gastric muscles, the curve sloping off to a maximum fall 5–25 sec. after a 10–20 sec. stimulus and gradually re-

turning to near the previous level during 10–80 sec. A latent period of several seconds before the relaxation began, and a partial or no immediate recovery of the tonus were the chief variations noted. Over 95 per cent of the stimuli which stopped respiration were accompanied by gastric relaxation, and 90 per cent of the positive responses of the stomach were so prompt and brisk that they were unquestionably due to the stimulus. Frequently the gastric tonus decreased with stimuli only sufficient to

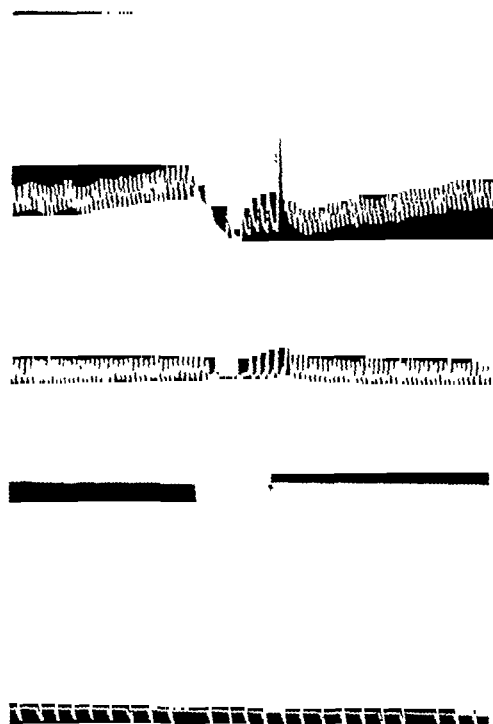


FIG. 4. Monkey No. 1. Tracings same as Fig. 3 except for absence of blood pressure curve. Stimulus 3 V applied to area shown in Fig. 2. Abrupt decrease in tonus of gastric muscle although no prolonged respiratory arrest appeared. In both Fig. 3 and 4 the prominent spike in the first and second tracings represents the deep breath appearing immediately upon cessation of stimulus.

slow and not stop the respiration, and infrequently gastric relaxation occurred at an even lower threshold than that for any inhibitory effect on respiration. Since the onsets of dilatation and subsequent contraction of the stomach were at times exactly coincidental with cessation and resumption respectively of the respiration, the possibility of the gastric responses being due to a mechanical artifact from absence of the inspiratory pressure of the diaphragm was considered. The facts that respiratory arrest also occurred with no change in the gastric volume, that relaxation of the gastric muscle occurred at times with stimuli which caused no respiratory change, that occasionally abrupt gastric relaxation occurred with a shorter latent period than that for respiratory alteration and that gastric volume usually returned to its previous level slowly in the manner of smooth-muscle contraction rather than promptly with the resumption of normal respiration, led us to conclude that the mechanical factor was at most a minor one in explaining the shifts in the curves of gastric response.

Accessory effects. The masticatory, glossal and deglutitory movements noted by W. K. Smith in his cats upon stimulation of the area inhibiting respiration were seen also by us. These movements occur in less than one-half of the stimuli inhibiting respiration, and we agree that they are more likely to occur when the anterior part of this area is stimulated. We also noted that stimulation of the gyrus orbitalis immediately adjoining the horizontal ramus of the sylvian fissure occasionally caused closure of the contralateral eye. None of these phenomena occurred during stimulation of the gyrus orbitalis in the monkey.

SUMMARY

From the orbital surface of the frontal lobe in both cats and monkeys an area in the gyrus orbitalis near the olfactory tract was found to give rise upon stimuli of 1-6 V to inhibition of respiration, rise of blood pressure and decrease in the tonus of the gastric musculature. The respiratory effect was obtained nearly always in the cat and always in the monkey; the elevations of arterial pressure were higher and more consistently present in the monkey; the inhibition of tonus in the gastric muscles occurred only infrequently in the cat but in the great majority of instances in the monkey.

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rents can be detected in muscles exhibiting the rigidity of the decerebrate state. He also devoted attention to the neck and labyrinthine reflexes which had recently been described by Magnus and de Kleyn, pointing out that their presence could be detected in animals with an intact nervous system (16).

During the third phase of Dusser de Barenne's scientific activities from August 2, 1914, to April 28, 1918, while serving as medical officer on active duty in the Dutch Army, he found time to study the mechanism of tonic contraction of skeletal muscle (19, 21), as well as the functional localization of sensory phenomena in the cerebral cortex (18). With J. Boeke, he published a paper on sympathetic innervation of skeletal muscle (25), and despite the heavy responsibilities of military service, he was able during this unsettled period to prepare ten important scientific papers.

Dusser de Barenne left the army just before the cessation of the first world war to accept an appointment as Lecturer and Privat Dozent in the Departments of Pharmacology and Physiology at Utrecht University, a post which he held from May 1918 until September 1930. During these years, he was in intimate contact with one of the foremost contributors to the literature of neurophysiology, Rudolph Magnus. He joined Magnus in a study of the physiology of posture (29), and also gave attention to the functions of the cerebellum; but his interests were catholic, for in addition to the subjects just mentioned, one finds papers on the action of insulin (42), on the metabolism of muscles during decerebrate rigidity (43), on the influence of the vagi on action currents of the diaphragm (39), and several papers on nystagmus (48, 61).

His most important contribution during these years came as a result of a visit in the spring of 1924 to the Laboratory of Sir Charles Sherrington at Oxford. There he studied the sensory symptoms which followed the local application of strychnine to the cerebral cortex of rhesus monkeys. The paper embodying these findings published in the *Proceedings of the Royal Society* in 1924 (vol. 96B, pp. 272-291) entitled "Experimental researches on sensory localization in the cerebral cortex of the monkey (*Macacus*)" is now a classic. It demonstrated for the first time the major functional subdivisions of the sensory cortex, i.e., the areas for the leg, arm and face; the paper was the first of an important series on functional localization in the cerebral cortex.

By this time, Dusser de Barenne had, by common consent, become the foremost of the younger generation of Dutch physiologists. With the deaths of Willem Einthoven and Rudolph Magnus, both in 1927, and the retirement of Hendrik Zwaardemaker, the three most important chairs of Physiology and Pharmacology became vacant in Holland almost simultaneously. Any one of them Dusser de Barenne would have filled with distinction, and he no doubt would have been called to one of them had it not been for religious restrictions in the Dutch universities, which were intolerable to a free-thinker of Dusser de Barenne's outspoken tendencies. And so it came

to pass that Holland allowed the United States to claim one of the most distinguished physiologists the continent of Europe has ever produced.

The three chairs just mentioned had all been filled by the autumn of 1928, and in the spring of 1929 Dean Winternitz travelled to Utrecht hoping to persuade Dusser de Barenne to come to the Yale School of Medicine. The International Physiological Congress was being held that summer in Boston (August 19-24, 1929) and the International Psychological Congress a week later in New Haven (September 1-4, 1929). Dusser de Barenne attended both meetings, and shortly after his return to Europe decided to come back to New Haven in a year's time to establish a research laboratory in the field of neurophysiology.

Professor Dusser de Barenne arrived in New Haven with his family on September 24, 1930, but since the extension of the Sterling Hall of Medicine in which his laboratory quarters were to be placed was then in process of construction he spent his first year in temporary quarters in the Brady Laboratory. His first collaborator at Yale was Clyde Marshall, with whom he described a release-phenomenon induced by isolating a focus of the motor cortex from adjacent cortical areas (66); his second collaborator was Stephen Brody, with whom he studied the effects of hyper-ventilation on excitability of the cortex (71). These two papers inaugurated a series of highly important studies on the functional organization of the cerebral hemispheres in primate forms. With David Koskoff he discovered the phenomenon of flexor rigidity (72, 81) in spinal cats (1931-33). Richard Wendt also carried out studies on conditioned reflexes in his Laboratory in 1933-35 and Dusser de Barenne often referred to the valuable improvements in systems of recording which Wendt introduced.

In 1934 a fruitful collaboration was begun with Warren S. McCulloch, who for six years has been his devoted and congenial colleague in research. Joined later by Leslie Nims, Carl Hovland and others, a team developed which demonstrated the reciprocal relationships between the activity of the cortex and its hydrogen-ion concentration. The research program inaugurated by Dusser de Barenne at Yale thus proceeded in the most logical manner from the release phenomenon discovered with Marshall to the effects on excitability in hyperventilation studied with Brody, to the new technique of laminar destruction of cortical cell layers with Zimmerman, the study of excitability cycles and the discovery of the phenomenon of "extinction" with McCulloch, and the pathological changes following laminar coagulation. The program culminated in the correlation of these phenomena with changes in the hydrogen-ion concentration (studied with Nims and McCulloch).

With these basic correlations established, it was possible to study two general problems: (i) the influence of one cortical area upon another, and (ii) the interaction between the cerebral cortex and the thalamus, and between the cerebral cortex and the basal ganglia. It is in the last phase of this work that Dusser de Barenne and his colleagues have been engaged

during the past two years. In 1939-40, with Percival Bailey, Hugh W. Garol, William E. Stone and Craig W. Goodwin, they had extended their studies to the chimpanzee.

In reviewing Dusser de Barenne's work, one becomes aware that there are few parallels in the history of physiology to his sustained productivity, especially during his later years. It culminated during the last ten in one of the most important series of physiological papers to emanate in a corresponding interval from the pen of one man.

In his physiological work Dusser de Barenne developed many new techniques such as the strychnine method for localization of sensory function, laminar coagulation for analysis of the cortical layers, and the adaptation of electrical techniques for study of the interaction of specific cortical areas. He will be remembered for his unyielding faith in the experimental method and for his utter intolerance of those who place the armchair ahead of the experimental table as a place for solving the problems of physiology—"never think, if you can experiment." He was a man of strong personality and strong loyalties, and in greeting a stranger he often seemed to regard him as a scheming, insincere fellow until he had proved himself otherwise. This accounted for his proverbial caution with visitors and for humorous anecdotes that his many friends enjoy relating. But once anyone had proved himself worthy of Dusser de Barenne's friendship, the attitude and the friendship never altered.

In his personal life, he was reticent and never discussed his personal affairs outside the ranks of his immediate family. He was widely read in music, in art and in philosophy past and present, and he was essentially a Kantian in his outlook. He had a number of heroes. Among these was Claude Bernard, whose portrait was always before him on his desk; another was Carl Ludwig; and the third was his Chief at Utrecht, Rudolph Magnus. These were men of action, men of experiment, who never allowed their deductions to usurp the place of experiment.

The School of Medicine at Yale will also remember Dusser de Barenne as a teacher. While he had no formal obligation to teach in the School of Medicine, he offered each year one or two electives, on the sense organs, or on special phases of the physiology of the central nervous system. Almost invariably he illustrated the lectures by experimental demonstrations into which he put much time and thought, and usually he ended these amusing and informative discussions in a fever heat of perspiration. The students responded with great warmth of appreciation, for these were lectures unique in their medical experience.

As an editor of this *Journal*, he rendered an incalculable service, originally in formulating its policies, and during the three years since it was conceived. His editorial judgments were wise and just and they were always set down in terse and unmistakable language; while he read all manuscripts carefully, he reached his editorial decisions so quickly that papers rarely

remained on his desk more than 24 hours His loss to the *Journal*, as well as to physiology in its broadest sense, is irreparable.

Ave atque vale!

J F. F.
R W G

June 10, 1940

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Dr. Dusser de Barenne left some ten additional papers in preparation.

HYPOTHALAMIC LESIONS AND PNEUMONIA IN CATS

WITH NOTES ON BEHAVIOR CHANGES

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DURING the course of a series of experiments in which the effects of extensive hypothalamic lesions in cats were studied, a large proportion of the animals succumbed to bronchopneumonia within the first postoperative week (1). It is our present purpose to report upon an investigation of this phenomenon.

METHODS

Forty adult cats were operated upon as follows: Each animal was anesthetized with nembutal, the skull trephined, and the Horsley-Clarke apparatus mounted (2, 3). An electrode was then inserted stereotactically so that 3 or 4 vertical punctures spaced 2 mm. apart in a rostro-caudal plane were made into the hypothalamus. At from 2 to 4 points in the course of each puncture a direct current of from 3 to 4 mA was passed for 40 sec. in order to coagulate the surrounding nerve tissue. In separate series of animals the lesions were placed as follows: The rostral lesions (5 cats) extended from the anterior commissure to the caudal margin of the optic chiasm; the middle (27 cats) from the caudal border of the chiasm (which was not destroyed) through the tuber cinereum into the mammillary bodies; and the caudal (8 cats) from the middle level of the mammillary bodies into the mesencephalon to

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transferred to a constant temperature room (92°F.), where it remained continuously except for daily periods of laboratory observation of from 30 to 60 min. at about 75°F. After from 1 to 24 days of observation the animal was autopsied and the brain and viscera removed for microscopic examination.

Eight of the 27 animals with middle hypothalamic lesions died before the 5th postoperative day from such causes as thrombosis of the circle of Willis, brain abscess or meningitis and were therefore eliminated from the series. The remainder (32 cats) are classified as (i) survivals, including cats which presumably would have lived indefinitely had they not been sacrificed and a small number which died 5 or more days postoperatively of causes other than pneumonia; and (ii) animals in which bronchopneumonia was found to be the cause of death. The 32 animals were therefore distributed with respect to site of lesion as follows: rostral, 5 cats; middle, 19 cats; and caudal, 8 cats.

RESULTS

Rostral lesions: Animals with such lesions often sought the investigator's attention and were responsive to fondling, although two proved unusually aggressive toward dogs and other cats. All 5 animals survived and none showed polydipsia, polyuria nor appreciable loss of temperature regulation.

Middle lesions—General behavior. The animals with these lesions were lethargic and had some degree of extensor hypertonicity for varying periods after operation. When left undisturbed, they usually showed no affective behavior and were equally indifferent to food, rats, and dogs. Nevertheless, they were able to defend themselves effectively with teeth and claws against

tube feeding and restraint, although movements were not as efficient nor as well integrated as those of normal animals. They responded to stroking by arching of the back, but purring could not be elicited. When subjected to exceptionally aggressive attacks by other cats or by dogs they defended themselves in the supine posture typical for combat in close quarters; when thus aroused they remained alert for a short time and forestalled further engagements by hissing, growling, and striking out with extended claws. When held upright by the nape of the neck with hind paws touching the floor, animals with middle lesions often tried to leap high into the air and to claw wildly until again set upon all fours, after which they ran aimlessly away. Several such animals, provided with a corner of coarse screening, repeatedly climbed as much as 4 feet above the floor and resisted all efforts to dislodge them, although they ultimately dropped off and once on their feet scampered away. After about a week the long-term animals of this group began to recover from their lethargy, to regain their temperature control and behave more nearly like normal animals. However, the other 16 lived only an average of 3.5 (range 1 to 8) days, and followed a gradually declining course characterized by increasing malaise, a rising temperature and the physical signs of widespread bronchopneumonia.

Temperature regulation. All animals with middle hypothalamic lesions were relatively poikilothermic, their body temperatures falling from 1.5 to 4.7°F. during 30 min. of exposure in the laboratory. Nevertheless, absolute poikilothermia did not occur, since no animal had a body temperature below 99.8° while in the incubator at 92°, whereas the lowest temperature recorded in the entire series was 91.4° in a cat which had remained 36 hours at a maximum temperature of 78°. Furthermore, twelve of the partially poikilothermic animals were able to respond to pneumonic and other infections with a fever of 104°, 7 above 105.5°, 4 of 107° and one as high as 108°. In general, the higher the fever the greater the rate of fall in body temperature when the animal was removed from the incubator. Shivering and abnormal panting occurred in 2 animals but no instance of neurogenic fever was observed.

Caudal lesions. Animals with such lesions manifested varying degrees of catalepsy and muscular hyperreflexia, but these diminished after the first postoperative week in the 6 animals which survived. One of the remaining 2 cats died on the 7th day after the experimental administrations of massive doses of benzedrine sulphate; the other, who succumbed to pneumonia after 7 days, was the only animal with a caudal lesion which was definitely poikilothermic. However, one of the surviving animals had a ("neurogenic"?) fever of from 103 to 107°F. for 11 days before sacrifice; at autopsy no explanation for the pyrexia could be found.

DISCUSSION

Etiologic factors in pneumonia. The strikingly high incidence of pneumonia in the cats with middle hypothalamic lesions led to the consideration of the following possible etiologic factors: (i) pre- or postoperative distemper,

(ii) effects of anesthesia; (iii) aspiration; (iv) septic pulmonary emboli from the operative or other infected site; (v) laryngeal obstruction; (vi) gastrointestinal disturbances; (vii) exposure to artificial extremes of temperature; (viii) hemorrhage about the brain lesion; (ix) cardiovascular collapse; and (x) loss of normal temperature regulation.

Distemper. Care was taken to select cats free from all types of infection, respiratory or otherwise. Only two animals developed postoperative upper respiratory infections and both survived.

Anesthesia. Light nembutal anesthesia (0.02 to 0.03 gm. per kg. intraperitoneally) was used in all operations and therefore remained a constant factor. Furthermore, in another series of over 200 recovery animals in which the same anesthetic was used in performing non-destructive hypothalamic operations, the eight-day mortality rate was less than 4 per cent, as compared to 85 per cent in cats with middle hypothalamic lesions.

Aspiration. Certain general measures were taken in all of the experiments to prevent aspiration. During operation the rear end of the cat board was elevated and for the next 24 hours, during which time aspiration was most likely to occur, the cat was strapped head lowermost into a specially constructed and tilted cage. All animals were maintained for 24 hours before and after operation solely by the intraperitoneal injections of 10 per cent dextrose in Ringer's solution, and feeding was not resumed until the animal could again swallow normally. All the animals with hypothalamic lesions coughed vigorously when a foreign body entered the trachea, indicating that this safeguard against aspiration had not been abolished. Further to control the possibility of the aspiration of saliva, enough atropine to prevent salivation (usually 6 mg.) was given to 5 cats about 30 min. before they were anesthetized and repeated until deglutition and coughing had returned to normal; nevertheless, 4 animals died of pneumonia and one of meningitis. Drainage of infectious material from the middle ears could also be eliminated as a direct cause of pneumonia inasmuch as only one animal in the group with middle hypothalamic lesions had a middle-ear infection, whereas 4 animals with otitis media* in the other groups did not develop pneumonia. Every precaution was taken to prevent aspiration during tube feeding; in one instance the milk mixture was known to have been aspirated, but this is the only animal in which subsequent microscopic sections of the lungs were positive to neutral fat stains. As a final control on the effects of tube feeding, 7 animals were maintained solely upon intraperitoneal injections of 10 per cent dextrose in Ringer's solution, yet 6 of them died of pneumonia.

Pulmonary emboli. Animals which died of infections that might have given rise to septic emboli were not included in the pneumonia series. Furthermore, the gross appearance of the pneumonic lungs did not suggest multiple septic infarction, but rather showed discrete or confluent bronchopneumonic foci which usually involved all the lobes. This was confirmed by

* The insertion of the Horsley-Clarke ear-cones damaged the middle ear to a certain extent in all experiments.

microscopic examination, which revealed no evidence of emboli or infarctions, but consistently showed intense vascular congestion, alveolar edema and peribronchial pneumonitis. Bacterial smears taken from the fresh specimens showed numerous medium sized, blunt, spore-forming, Gram-positive rods and occasionally large Gram-positive diplococci. Unfortunately, more complete bacteriological studies were not made.

Laryngeal obstruction. All the animals vocalized normally after recovery from the anesthesia, indicating unimpaired laryngeal function.

Gastrointestinal disturbances. Keller (4) has commented upon the importance of constipation in the mortality of his operated animals. Few of our cats were constipated, and when they were, cascara sagrada was given within 36 hours. Postoperative diarrhea occurred in only a few and was not related to the incidence of pneumonia. In no instance was there evidence of infection of the peritoneum or of the gastrointestinal tract.

Exposure to extremes of temperature. None of the animals in this series was subjected to cold- or hot-box experiments. The maximum change of temperature to which any animal might have been exposed was 23°F.; more commonly it was only 17°.

Brain hemorrhage. Keller (4) attributed some of the mortality in his operated animals to hemorrhage in the region of the tuber cinereum. This complication was apparently avoided in our experiments by the use of the Horsley-Clarke instrument, since all extravasations were microscopic and confined to the area of electrocoagulation.

Cardiovascular disturbances. The possibility of progressive failure of cardiovascular or respiratory control as a result of the destruction of hypothalamic centers was not definitely excluded in these experiments, although such failures were not observed either in the recovery animals or in a separate series of experiments in which the hypothalamus of anesthetized animals was serially destroyed by electrolysis while graphic records were made of the respiration and blood pressure (1). Moreover, when 6 animals in our survival group were re-anesthetized and subjected to acute experiments from 7 to 22 days after the original lesions had been placed, none showed an abnormal blood pressure tracing or respiratory curve.

Failure of temperature regulation. Persistent hypothermia occurred in only 7 of the 19 animals with middle hypothalamic lesions, but all pneumonic animals with middle lesions were markedly poikilothermic, i.e., showed a significant fall in body temperature upon removal from the incubator. On the other hand, 3 poikilothermic animals survived from 8 to 18 days and one cat with a caudal lesion had a temperature as low as 97° during 24 hours in the laboratory, yet showed no lung involvement. It was apparent therefore, that disturbances of temperature regulation were important but not determinative factors in the incidence of pneumonia.

Previous observations. The spontaneous and reactive behavior of these animals with rostral and caudal hypothalamic lesions was essentially the same as that described by Teague and Ranson (5) and by Ingram, Barris, and Ranson (6) respectively. The "large rostral" and "large caudal" lesions of Clark, Magoun, and Ranson (7) were somewhat

more extensive rostrocaudally and mediolaterally than ours and probably for that reason were followed by a significantly higher mortality. Also, their incidence of postoperative diarrhea was higher than ours, whereas we observed a considerably greater frequency and degree of fever in response to infection. The high mortality in our animals with middle hypothalamic lesions confirms the observation reported by Beattie, Brow, and Long (8) and by Clark, Magoun, and Ranson (7). The latter authors report a 100 per cent mortality following similarly placed but usually less extensive lesions and state that the "... lungs were found congested or consolidated in each case." Nungester and Klepser (9) showed that exposure of rats to cold, presumably lowering their body temperatures, reduced or abolished closure of the glottis during deglutition, thereby permitting aspiration of oral and nasal contents. Whether or not such a dysfunction of the glottis occurred in our poikilothermic cats was not specifically investigated, but that it alone could account for the high incidence of pneumonia is rendered doubtful by the persistence of an active cough reflex and by the precautions taken against aspiration. What relationship, if any, exists between the pneumonia following middle hypothalamic lesions and the "vagus pneumonia" or "neuropathic pulmonary edema" produced by bilateral cervical vagotomy (10-14), or the pulmonary congestion following large doses of eserine (15) is also not determined; however, the work of Schafer (16) indicates that cats can survive bilateral cervical vagotomy provided laryngeal obstruction is avoided. Our cats vocalized normally, indicating undisturbed laryngeal function. Nevertheless, in the 5 animals with the middle hypothalamic lesions which succumbed within 24 hours after operation the marked pulmonary congestion and alveolar edema were strikingly similar to the changes found after double vagotomy.

The severe disturbance in temperature regulation in our animals with middle lesions appears to be definitely related to the high incidence of pneumonia in the group. In this respect, Locke (17) observed that rabbits which were slow to regain their normal body temperature after having been chilled to 95° showed a lower resistance and a higher mortality after the intravenous or intradermal inoculation of pneumococci than did rabbits which regain their normal temperatures rapidly. Similarly, Robertson (18) found it advantageous to lower the temperatures of his dogs in order to facilitate the experimental production of pneumonia by the intrabronchial inoculation of pneumococci. However, a subnormal body temperature could not have been the decisive factor in the mortality rate since less than half our pneumonic animals were hypothermic. Furthermore, 2 animals with subnormal temperatures survived, an observation in accord with the findings of Clark, Magoun, and Ranson (7) who also reported a number of hypothermic cats among their survivals. Neither was poikilothermia itself an absolute factor, inasmuch as 3 of our markedly poikilothermic animals did not develop pneumonia. As stated, therefore, the disturbance of the temperature regulatory mechanism is probably of etiologic importance in the pneumonia which follows middle hypothalamic lesions. The precise mechanism which must be sought in view of the fact that 89 per cent of the animals with hypothalamic lesions died of some type of infection whereas only 14 per cent, some of these factors may be concerned with a loss of the animal's resistance to sepsis.

This study throws no light upon the problem of precise localization of the neural structures involved in the regulation of body temperature, since only extensive lesions were made. However, our findings confirm those of many other workers (5, 7, 19-32) that the nervous mechanisms which prevent an excessive fall in body temperature lie somewhere between the rostral margin of the tuber cinereum and the caudal borders of the mammillary bodies.

SUMMARY

1. Forty cats were operated upon in 3 series. Bilaterally symmetrical lesions were placed in the rostral (5 cats), middle (26 cats), and caudal (9 cats) regions of the hypothalamus.

2. Characteristic changes in the motor behavior and the emotional responses of the animals are described.

3. All animals with rostral lesions (anterior commissure to optic chiasm) survived. Sixteen of the 19 animals with middle destructions (chiasm into mammillary bodies) succumbed to bilateral bronchopneumonia in 1 to 8 days. Two of the 8 animals with caudal lesions (mammillary bodies and portions of the mesencephalon) died of pneumonia on the 7th day.

4. Animals with rostral lesions retained the ability to prevent an abnormal fall in body temperature; those with caudal lesions showed occasional disturbances of temperature regulation; whereas animals with middle lesions showed marked poikilothermia. All animals responded to infection with fever, but those with middle lesions could not maintain the hyperthermia in the presence of fluctuating environmental temperatures. A close correlation existed between the degree of poikilothermia and the incidence of pneumonia but important exceptions occurred.

5. The following possible contributory factors were studied and found to be of little or no significance in the etiology of pneumonia in the animals with middle hypothalamic lesions: distemper, anesthesia, basilar hemorrhage, peritonitis, aspiration, laryngeal obstruction, pulmonary emboli, gastrointestinal disturbances, and exposure to extremes of temperature. Destruction of the central region of the hypothalamus therefore causes a high incidence of fatal pneumonia in cats, but the specific etiologic factors in this relationship require further investigation.

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PROGRESSION MOVEMENTS ELICITED BY SUBTHALAMIC STIMULATION

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THE VENTRAL part of the thalamus is generally accredited as a participant in two groups of functions, and two rather ill-defined anatomical subdivisions may accordingly be recognized. Its rostral half, and that part of its caudal half which lies next the midline, are included in the hypothalamus. The lateral part of its caudal half, ventral to the caudal part of the dorsal thalamus, is the territory of the subthalamus. In recent years the hypothalamus has come to be recognized as the seat of a high level of control of autonomic function and many of its activities have been demonstrated, but the functional complexities of the subthalamus have been more difficult to elucidate.

From the nature of its fiber connections (Jakob, 1925; Morgan, 1927, 1928; Papez, 1938) it is evident that the subthalamus is a part of the striatal system, which is, largely on the basis of clinical observations, commonly classed with the motor systems of the brain. Thus far no unequivocal proof, either clinical or experimental, has been adduced to show that any definite motor function is restricted to the corpus striatum, but the results of several recent investigations indicate that motor functions may be demonstrable in the subthalamic part of the striatal system. Since the subthalamus is not sharply delimited anatomically from the hypothalamus, some difficulty in separating the functions of the two parts of the ventral thalamus may be expected. Ectors, Brookens and Gerard (1938) stimulated the cut surface of the brain of the cat exposed by making a parasagittal section and removing about half the brain, and obtained running movements from the region of the ventral thalamus. Masserman (1938) inserted an electrode into the ventral thalamus of the cat with the aid of a Horsley-Clarke stereotaxic instrument, and observed running movements of the front legs during the passage of current to make electrolytic lesions. In the experiments of Rioch and Brenner (1938) stimulation of the rostral part of the hypothalamus was followed, after a long latent period, by violent running movements. None of these investigators determined the neurological localization of the region which was active in initiating the locomotor movements. (See also Hinsey, 1940, which appeared after this paper went to press.)

METHOD

Studies of the results of electrical stimulation of the ventral thalamus were made in 18 cats. Most of the animals were lightly anesthetized with nembutal, the aim being to use the smallest dose that would eliminate practically all spontaneous movement. A dose of about 25 mgm. per kg. was usually sufficient; in some cases a smaller amount was given first, and additions were made until spontaneous movements stopped. In each experiment the calvarium was opened with trephine and rongeurs, the Horsley-Clarke instrument was affixed to the head, and the cat was suspended in a cloth sling hung by six strings from a wood

frame, so that the feet were either free or barely touching the table. A bar across the top of the frame held the carrier of the Horsley-Clarke instrument, which was the sole support of the head.

All stimulation was unipolar. The indifferent electrode was a steel probe inserted handle first into the rectum. The active electrode was a No. 24 nichrome wire coated except at its sharpened tip with insulating varnish and placed at any desired point in the brain by adjusting the position of the needle carrier of the Horsley-Clarke instrument. Alternating 60 cycle current of 6 v. was obtained from the house circuit with a transformer and was passed through a 100 Ω radio volume control into the electrode circuit, which was closed and opened with a manual key switch. This apparatus was found to be far superior to the Harvard coil which was tried in several preliminary experiments. Stimulating voltage could be varied by means of the dial on the volume control, which was scaled from 0 to 150, so that, for example, a reading of 25 indicated about 1 v. in the electrode circuit (assuming the resistance of the electrode circuit to be considerably greater than 100 Ω). Electrolytic lesions made in some animals, using the same electrodes and current of 2 to 5 mA. derived from a 45 v. battery and controlled by a variable resistor and a 1 to 15 mA served as obvious marks against which the vertical (depth) readings of the needle carrier could be checked.

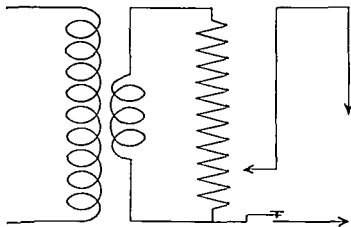


FIG. 1. Wiring diagram of the stimulator.

At the conclusion of each experiment the head of the cat was perfused through the carotid artery with 10 per cent formalin containing 2 per cent glacial acetic acid, and the brain was removed and immersed in the same fluid. After nitrocellulose embedding the brains were cut in 50 μ sections in planes as nearly as possible parallel to the frontal plane of the Horsley-Clarke instrument, and every 8th section was stained with thionin and mounted on a slide. Allowing for 20 per cent shrinkage in the process of dehydration, the sections in the mounted series were 0.5 mm. apart. In some cases parallel sets of Weigert stained sections were prepared. The positions of the needle tracks as seen in the sections were then marked on a set of form drawings of the basal ganglia and compared with the results of stimulation as recorded in the protocols. In each needle track the actual depth of the needle point for each vertical reading was determined by reference to the zero horizontal plane on the form drawings, the position of which was determined in animals in which lesions were made.

RESULTS

In most of the experiments stimulation in the region of Forel's field (Fig. 2, rsb) caused alternating movements of the legs similar to those made by the cat in the normal act of walking. Many variations and inconsistencies were observed, as is common in stimulation work, but only repeatedly recorded responses will be reported.

Localization. The response was localized both topographically and temporally, quite sharply in some animals. Best results were usually obtained when the needle point was 2 to 3 mm. lateral to the midline, 7 to 8 mm. rostral to the zero frontal plane and 3 to 4 mm. below the zero horizontal plane of the Horsley-Clarke instrument. Stimulation of points 1 or 2 mm. dorsal, ventral, medial, lateral or rostral to the point giving the strongest response was entirely ineffective in some cats, but produced results in others if the voltage was increased.

SAMPLE PROTOCOL

Cat. 168. Operated Mar. 31, 1939. Weight 2200 g.; nembutal 15 mgm./kg. (unusually small dose). The distances in mm. rostral to the zero frontal plane (A) and lateral to the midline (L or R) for each needle track are given once at the beginning of the series of records of results observed on stimulation at various distances above (H) and below (H-) the zero horizontal plane in that needle track. Usually several trials at various voltages were made at each vertical setting of the needle. Autonomic responses have been deleted. Voltage is indicated for some points, using the letter P (P10 means 10/150 of 6 v., or 0.4 v.). Responses designated as follows: RF, RH, LF, LH: right front leg, right hind leg, etc.; H: head; R: caudal end of trunk (rear); r or l: moves or turns to right or left; fl: flexion; fo: moves forward; X: walking movements of all 4 legs; XX: running of all four legs; V or VV: walking or running of front legs; sl: slight; C: tegmental response, head and rear to right, trunk to left; \mathcal{O} : same, head and rear to left; no: no response. Parts of this protocol have been deleted. The positions of the needle tracks on the right side which are included below are shown in Fig. 2.

10 A.M.: frontal cortex exposed and stimulated.

2 P.M.: stimulation of basal ganglia.

A10R5H5,3;no; H1,O: LF&LHfl; H-2: LF&LH run; H-4: V1 LF better, & LH walks; H-5: LF back RF pawing; H-4 repeated: VV. A10L6H5;no; H3: RF&RHfl; H1,O: RHfl&HR; H-3: RF&RH pawing; H-4,-5;no.

A10L5H5,3;no; H1,-1: RF&RHfl; H-3: RF runs; H-4: Hr&lapping.

A4L3 H7,5;no; HO: \mathcal{O} &LFfl&RFext; H-1: \mathcal{O} ; H-2 P10: XX; H-3: X at P10, no at P25; H-4 P25: X; H-5 P15: RF&RH run, chewing and lapping (no LF&LH even at P35).

A4R3 H5,3;no; HO: H1 Rr RFfl; H-2 P10: all run except LH; H-1.5 P1: XX; H-4 LHfl; H-5 P25: panting, LF runs; H-6 P25: all run except LH, but front legs get in each other's way.

A4R2 H3,2 P20: eyes up; H1: RFfo; HO P15: RFfl&LFextRrHl; H-1: same, but runs when current cut to P10 or lower, tail swings in circle; H-2, -3: C; H-4: LF&LHfl.

A4R4 H5,3,1;no; HO, -1, -2 P25: RFfl&LFextRr; H-3 P5: XX; H-4 P25: LF runs; H-5, -6 P10: all run except LH (unusual result); H-7 P20: V; H-8: LF&LH run.

A8R2 H5,3;no; H2,1,0,-1 P25: C&LFfl; H-2: same and runs after current off; H-3 P10: X if started at P20 or if front leg is given a push; H-4 P10: X but stops while current is still flowing.

A11L9H5,3,1,0,-1,-2,-3;no; H-4: chewing.

A4L1 Eye movements and tegmental response, no walking. Further cortical stimulation and small lesion made in right motor cortex. Incision closed; cat returned to cage.

Second operation April 26, 1939.

A8L4 H5,3;no; H1,2: RFfl; H-1;no; H-2,-3: RFsl fo Hr; H-4,-5: lapping, panting.

A8L2 H5,3;no; H2,1,0: RFfl; H-1;no; H-2,-3,-4: RFfoHr; H-5: lapping.

A8R2 H5,3,1;no; HO,-1 P17: V; H-2: C; H-3 P10: V; H-4 P13: V; H-5 P24: V; H-6 P30: irregular jerk.

A8L1 H5,3,2,1;no; HO,-1: RFfl; H-2: \mathcal{O} &RFfoLFext; H-3 P10: VrHr; H-4: same at P17; H-5,-6;no.

A8R1 H5,3,2,1,0,-1,-2,-3;no; H-4 P35: VV; H-5 irregular jerk; H-3 P35: VV.

A8R3 H5;no; H3: very sl V; H2: trunk left; H1,0: LFsl fl; H-1,-2 P14: V1Hl; H-3 P13: X; H-4 P16: V; H-5 P29: V.

A8L3 H1: RFfl; HO;no; H-1: sl X; H-2: X; H-3: RFfo; H-2;no; H-1: sl V; HO: RFfl.

A10R7H5,1,3;no; H2: LHfl; HO P15: X continues after current off; H-1: LHfl; H-2,-3,-4;no; replaced needle because of damaged insulation.

A10L7H5: Hr; H3,1,0;no; H-1,-2: RHflHr; H-3,-4,-5,-6;no.

A10L9H5,3,1,0,-1,-2,-3,-4,-5,-6,-7,-8;no; same at A10R9;no.

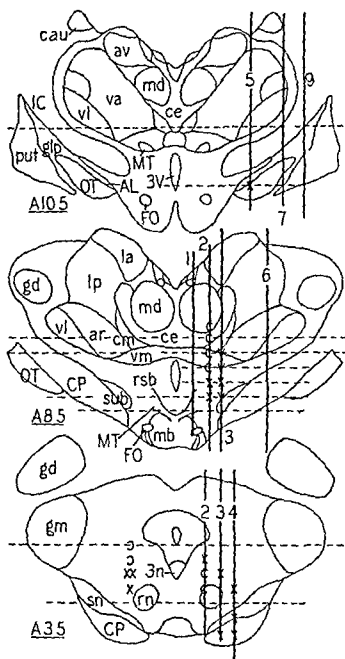
A9R6 H5,3,1,0,-1;no; H-2: LHfl; H-3: LFsl fl; H-4,-5,-6,-7;no.

A8R2 H-4 P15: V; H-5 P50: V; H-3 P17: V.

A9L5 H5,3,1,0;no; H-1 P18: V; H-2,-3,-4: RHflHr; H-1: RF&RHfl; HO P15: V.

Several punctures were made in the midbrain (A4) and walking responses were obtained from both sides.

FIG 2 Outline drawings of sections through the rostral (A10.5), middle (A8.5) and extreme caudal (A3.5) parts of the thalamus, 3 times natural size. Some of the needle tracks made in one animal, and responses obtained at points along them, are marked on the right side (see sample protocol). Needle tracks at A8 and A9 are marked on the drawing at A8.5. The number beside each needle track gives its distance from the midline in mm. The zero horizontal plane (HO) and a plane 4 mm below it (H-4) are marked by interrupted lines on each drawing, and in addition, planes 1, 2, 3 and 5 mm below the zero plane are shown at A8.5. Points at which progression movements were obtained are marked X if all four legs were involved, V if only the front legs moved. Points at which the tegmental response was elicited are marked by a C if the trunk was bent so as to be concave to the right, by a reversed C if the convexity was to the right. Labels: AL, ansa fenticularis; cau, caudate nucleus; CP, cerebral peduncle; FO, fornix; glp, globus pallidus; IC, internal capsule; mb, mammillary body; MT, mamillo-thalamic tract; OT, optic tract; put, putamen; rn, red nucleus; rsb, reticular subthalamic nucleus of Forel's field; sn, substantia nigra; sub, subthalamic nucleus of Luys; 3V, third ventricle; 3n, oculomotor nucleus. Nuclei of the dorsal thalamus: av, anteroventral; ce, central; cm, centrum medianum; gd, lateral geniculate body; gm, medial geniculate body; la, lateral anterior; lp, lateral posterior; md, medial; va, ventral anterior; vl, ventrolateral; ar, arcuate; vm, ventromedial.



Nature of the response. Rhythmic movements of the legs began within 2 or 3 sec. after the stimulating circuit was closed. They were ordinarily smoothly executed from the start, but occasionally there was a brief period of confusion before the regular alternation was established. Frequently the first steps were small ones, but as the current continued to flow the amplitude of the excursions of the legs increased to maximum in the course of 5 to 10 sec. When the response was confused or submaximal at its beginning it was usually apparent that the homolateral forelimb was responding best and was setting the pace. Once the maximal response was in progress, well coordinated walking movements could be kept going for more than a minute by continual stimulation, but no attempt was made to determine the upper limit of duration of effective stimulation. When the stimulus was stopped the movements ordinarily ceased promptly, but if the anesthetic was unusually light they continued several seconds longer. The excursions of the front legs were often in a plane oblique to the longitudinal axis of the body,

so that if the cat had been free he would have progressed forward and away from the stimulated side.

Incomplete responses. In some cases the hindlegs responded with movements of lesser amplitude than those of the forelegs, or did not move at all. Attempts to demonstrate the existence of separate points from which "two-legged" and "four-legged" walking could be obtained were unsuccessful. When only the front legs moved it was often apparent that the amplitude of the excursions was greater homolaterally than contralaterally. At times walking movements were performed by three legs while the activity of the contralateral hindleg was almost imperceptible. Three-legged walking in which the homolateral hindleg lagged was elicited also, especially by stimulation near the cerebral peduncle.

Effect of voltage variation. To elicit locomotor movements from the point of best response it was in most cases essential that the stimulus be neither too weak nor too strong. The rate and amplitude of the movements could not be controlled by changing the voltage, since supra-threshold stimuli evoked the tegmental response (see below) or irregular jerking movements. The voltage requirement varied in different cats from 2 to 15 on the volume control dial (0.1 to 0.6 v.). In those animals in which the progression response was not sharply localized and could be produced by stimulating points 1 mm. or more removed from the focal point in the subthalamus, effective stimulation at such points was in the range from 12 to 35 on the dial (0.5 to 1.4 v.), somewhat less precision in voltage regulation was required, and the movements were slower than those elicited by stimulation at the focal point.

Effect of depth of anesthesia. Accuracy was hardly less important in administering the anesthetic than it was in regulating the stimulating voltage. If the anesthesia was too profound no walking movements could be elicited. If it was too light, spontaneous movements interfered with observation of stimulation and the cat continued to move its legs after the stimulating current was stopped. Poor localization seemed to be associated with unduly light anesthesia. The locomotor movements obtained by stimulation at the point of best response were rapid in some animals; the legs moved more slowly, and sometimes rather stiffly, in cases in which the anesthesia was slightly deeper.

Stimulation of other regions. Walking movements were elicited in some animals by stimulation of a region extending caudally from the point of best response. The levels from A5 to A7 (not illustrated) have not been completely studied, but at A6 the locomotor zone seemed to be slightly more ventral and nearer the midline than it was at A8. At A4 it was found in the tegmentum dorsolateral to the red nucleus. Locomotor responses were occasionally obtained from the region of the ansa lenticularis and at times from the globus pallidus and putamen, but these were inconsistent. Walking movements could be elicited by stimulation of the internal capsule and cerebral peduncle by using voltages greater than those required to obtain the subthalamic response. Capsular progression movements differed from the sub-

thalamic response in that they were characterized by a tendency of the homolateral limbs, especially the hindleg, to lag behind.

Single limb movements. With the needle point in the subthalamus 1 or 2 mm. from the point at which locomotor movements could best be obtained, the response to minimal stimulation frequently was a single caudally directed movement of the homolateral forelimb. This response also occurred at times with the needle in the locomotor region, if the voltage was slightly too low for progression. Stimulation in the dorsal part of Forel's field (A8L or R3H-1 or -2) occasionally evoked flexion of the homolateral foreleg. The response of the ventral nucleus of the dorsal thalamus was flexion of the contralateral fore- or hind-leg, executed more deliberately than the contralateral flexion seen on stimulation of the internal capsule or cerebral peduncle.

Tegmental response. Stimulation near the red nucleus commonly evoked the tegmental response as described by Hinsey, Ranson and Dixon (1930), which consisted of bending of the rostral and caudal parts of the body toward the stimulated side. Curvature of the trunk was maintained for the duration of the stimulus, but some of the various limb movements which accompanied the response were rhythmic or clonic. In the subthalamus the region immediately dorsal to the locomotor point often gave the tegmental response. At times rhythmic pawing movements of the legs were superimposed upon the tegmental posture and it was difficult to separate the two responses. Slight reduction in voltage seemed to be the best procedure to eliminate the tegmental posture and produce uncomplicated progression movements. At certain points, particularly near the cerebral peduncle at A4, bending of the rostral and caudal parts of the body away from the stimulated side was occasionally seen (see Mettler *et al.*, 1939).

Autonomic responses. Since the locomotor point in the subthalamus is immediately adjacent to the hypothalamus it is not surprising that autonomic responses were often observed (Ranson and Magoun, 1939). Pupillary dilation commonly accompanied the walking response. No attempt was made to correlate the records and localize the regions from which autonomic responses were elicited.

DISCUSSION

The promptness, smoothness and regularity of the locomotor movements produced by subthalamic stimulation are evidence in themselves that the subthalamus contains a specific mechanism which governs the alternation of the legs in walking. It is hardly possible that the effect observed was caused by spread of current to pyramidal or other neighboring structures, since (i) the response was weakened or abolished by misplacement of the electrode in any direction except caudally, (ii) the strongest responses to subthalamic stimulation were homolateral, and (iii) the walking response was obtained in the subthalamus with stimulation of lesser strength than was needed to elicit rhythmic alternation from the internal capsule. Probably the activa-

tion of progression movements which can be produced by stimulation of the cerebral cortex or cortical fibers in the internal capsule (Tower, 1936) is effected through direct or indirect connections to the subthalamus. Attempts to verify Wilson's (1924) observation of walking movements on stimulation of the dorsal thalamus were uniformly unsuccessful. Except for contralateral limb retraction and turning of the head and eyes to the side opposite that stimulated, no somatic movements were consistently obtained by dorsal thalamic stimulation.

The locomotor point appears to be in Forel's field at the level of the subthalamic decussation, dorsal to the mammillary body and near the subthalamic nucleus of Luys. Hinsey, Ranson and McNattin (1930) found that cats in which this region had been separated from the more caudal parts of the brain stem by transverse section were unable to walk, while others with transections rostral to the mammillary bodies were not so disabled. The principal neurological relationship of the subthalamus is its reciprocal fiber connection to the corpus striatum, and occasional observation of walking movements in response to striatal stimulation indicates that the globus pallidus may normally activate the locomotor center in the subthalamus. But since experimental destruction of the corpus striatum (Morgan, 1927) does not incapacitate an animal for walking, the essential center must be in the subthalamus.

From the results thus far obtained, it seems reasonable to conclude that the subthalamus directs the order of movement of the limbs in walking by impulses which descend through the tegmentum dorsolateral to the red nucleus. That some mechanism other than the pyramidal and rubral systems must be capable of managing the act of progression was demonstrated by Evans and Ingram (1939), who found that a cat with lesions of its pyramidal tracts and red nuclei regained the ability to walk after a short period of disability. The nature and localization of the response to subthalamic stimulation seem to show that, at least in the cat, the descending tract from the subthalamus plays the leading role in regulating the alternation of the limbs. Since the feet were hanging free, there was, however, no evidence that the subthalamus has any direct control of the mechanisms responsible for bearing the body weight. Although under the conditions of the present experiments the reflex arcs normally activated by contact of the footpads were not in use, it is probably true that in directing the order of movements the flow of impulses from the subthalamus was materially assisted by crossed extensor and other spinal reflexes.

Since the depth of anesthesia affected both the degree of localization and the rate of execution of the response, it is quite probable that further variations may be found when the effects of subthalamic stimulation are investigated under different anesthetics.

SUMMARY

In cats anesthetized with nembutal, alternating movements of the legs

similar to those of normal walking and running were elicited by 60-cycle alternating current stimulation of the subthalamus in the region dorsal to the mammillary body. The sharp localization and low threshold of the response indicate that the subthalamus contains a specific center which directs the order of movement of the legs in locomotion.

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ELECTRICAL ACTIVITY OF THE LATERAL GENICULATE OF CATS FOLLOWING OPTIC NERVE STIMULI*

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TO ASCERTAIN the relation of the form of stimulus arriving over the visual pathway to the resultant cortical activity, we have recorded by means of the oscillograph responses from the vicinity of the dorsal nucleus of the lateral geniculate body in the cat. In order to locate the sources of such responses, all experiments have been controlled histologically, that is, the paths of all needle electrodes have been traced in serial sections 20 mm. thick. At critical depths of insertion, with needles insulated to within 0.5 or 1 mm. from the points, electrolysis at 1 mA. for a minute or less has served to identify the structures led from.

Responses were initiated by single or repeated condenser shocks or brief galvanic currents delivered through a rotating interruptor to the optic nerve. Nembutal, dial, ether, and magnesium sulphate were used as anaesthetics, the animals being usually maintained at a level where a single maximal shock caused no protest, but shocks repeated at 1 per sec. intervals aroused the animals (presumably by stimulation of pain fibers in ciliary nerves). During the preparatory operation ether was administered by tracheal cannula. A suitable level is maintained for experiment by, for instance, 0.2 to 0.4 cc. nembutal per kg., when 0.5 to 0.7 is required for a surgical anaesthetic in cats. Operations involved two procedures: first, removal of the eye and its muscles, without destroying the blood supply of the optic nerve by too clean dissection, a successful preparation being considered one in which a stimulus of 0.1 to 0.3 v applied for 0.5 msec. induced a threshold response; and second, exposure of the opposite cortex, with removal of its lateral aspect in some cases to expose the geniculate. If the retinal vessels of the strip of tissue left as a handle to the nerve cease bleeding, the nerve threshold rises promptly and the nerve soon ceases to respond at all. Apparently the venous return is destroyed in this operation. Needle electrodes may be employed to stimulate the nerve without removal of the eye, but with less precise control of response. Removal of the cortex damages the geniculate, probably through interfering with its circulation, to the extent that facilitation by the first of two shocks at the geniculate level had not been observed in such preparations. Needles thrust through the ectosylvian cortex into the geniculate region obviate the necessity of cortical operation.

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RESPONSES OF OPTIC NERVE, OPTIC RADIATION, AND
CORTEX TO OPTIC NERVE STIMULATION

It has been shown previously in the rabbit (Bishop, 1933b) that the optic nerve response to a maximal electrical stimulus includes two potential spikes, with indication of a third slower wave. It has also been stated that the fibers involved in producing the first of these spikes, that is, the group of larger diameter, were the only ones which activated the cortex (Bishop and O'Leary, 1938), and that fibers of the second group passed without synapse toward the colliculus. Allowing for some degree of overlap of these two size groups, this finding is confirmed in the cat. Further, the third smaller wave just behind the second in conduction rate can often be picked up, and a fourth, slow and temporally dispersed, is unmistakably present in favorable leads. (Fig. 1.) Second, third, and fourth groups of fibers can be traced past the geniculate. The second is followed by a prominent response from the pre-tectal and adjacent regions, the fourth causing a large response from the colliculus. The thresholds of these fibers correspond roughly to their conduction rates, the fourth group having the approximate rates of C fibers in peripheral nerves. (A much more prominent wave appears in this relative position in the frog optic nerve record, Bishop, *l. c.*)

In assigning potentials to structures, consideration must be taken of the fact that active tissue embedded in the (relatively) inactive mass of the brain will induce flows of current, and therefore potential differences, at some distance from the source. Some of the criteria for analyzing records of such diffused potentials have been discussed (Bartley, O'Leary and Bishop, 1937), and the conclusions arrived at below concerning the responses of specific structures are our best inferences based on what principles we can set up. That our principles are at present inadequate is evident from the circumstance that we cannot interpret some of our records, in which cases we can only fall back on the assumption, not conclusively established, that activity in any nervous tissue is accompanied by relative negativity of the active region. Aside from general physical considerations, chief reliance can be placed upon the assumptions that, *other things being equal*, the closer the one of two electrodes is to an active tissue, the higher will be the potential recorded from that tissue; that as an electrode is pushed through or past an active structure the most abrupt changes in the potential will occur just as the electrode passes through a plane containing the structure in question and the other electrode; and that if the electrode strikes and injures active tissue, it may serve as a dead-end lead, with consequent relative positivity of its immediate vicinity. The all too prevalent difficulty is that other things are not always equal.

Facilitation. It is certain that to one volley of impulses in the optic tract, the radiation responds with a single volley. To a threshold volley, a response of the radiation cannot be recorded. With increase of strength of single stimuli, the response of the radiation builds up more rapidly than does the response of the tract, *above the threshold for radiation response*. The second of

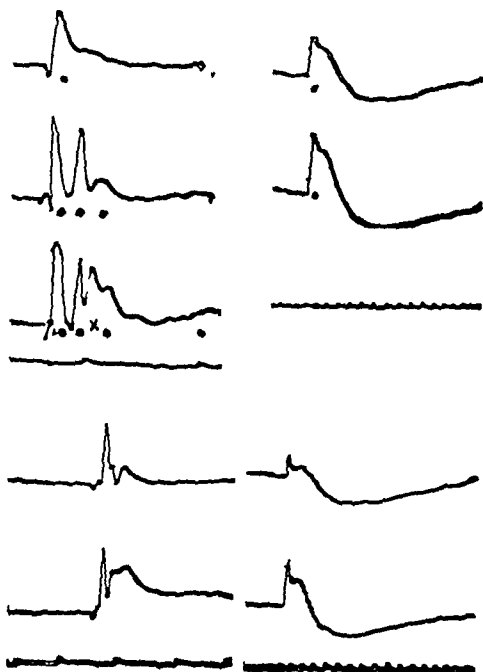


FIG. 1

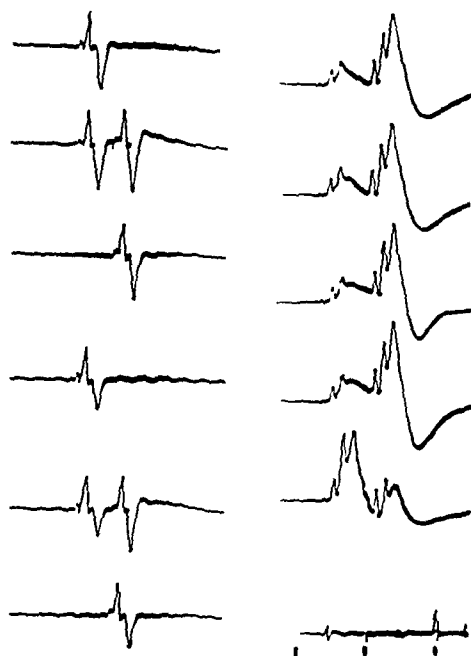


FIG. 2

FIG. 1. Potentials assignable to optic tract. Left, first 3 records, strengths of DC stimulus in ratio of 1:4:15. Dots below indicate the four waves, an extra spike in the third record X being a repetitive first spike. Time 5 msec. Leads from needle electrodes in tract near geniculate and in wall of ventricle. Right, slow waves following radiation response, leads from dorsal nucleus to wall of ventricle. The first tract spike and radiation spike are included in the highest deflection (dot), the slightly lower slow wave extending from its falling phase indicating negativity at the cell region. Time below 5 msec. intervals. Left, lower, first spike of tract up, radiation spike triphasic. The second tract wave (below) appears just behind and overlapping the radiation spike. Right, slower records at stimulus strengths of records to left. Compare the triphasic form of the radiation spike with the diaphasic form of the slow potential. The triphasic form is the result of the impulse travelling *past* one electrode. The implication is that the slow wave does not propagate. Time 5 msec. intervals.

FIG. 2. Cat, light nembutal anaesthesia. Left column, leads from tract to radiation, the first, paired, and second responses; 2.5 msec. interval between members of pair; upper series of 3, each record after a rest interval of at least 5 sec. Lower 3, each record one of a series repeated at 1 per sec. The initial or tract spikes are all equal. The radiation spikes are all lower in the repetitive series, except for the second of paired responses. This has the amplitude of single responses, while in the upper series, the second of the pair is even higher than any single response. All stimuli above maximal for first tract spike. Note summation of positive after-potentials in paired records. Right column, read from bottom up. Leads radiation to surface of lateral gyrus of cortex, 3 msec. intervals between pairs of shocks, first 5 pairs of a series, at intervals of 1 per sec. In first responses of each pair, radiation spike decreases after first record, but cortical response decreases much more, the later responses more than the earlier. In second responses of pairs, radiation spike of first record is higher than in first response, and remains so in subsequent records. Records after the first show marked facilitation, especially of diaphasic slow wave. Increase of stimulus strength did not alter this picture, but increase of interval between shocks of pair did. Time 5 msec. intervals.

two stimuli properly timed, both being "maximal" for the tract, may induce a larger response of the radiation than any single stimulus, and also below maximal, the second of two stimuli is considerably more effective than one alone. (Fig. 2A). All these findings indicate that facilitation in the sense of spatial and temporal summation is operative even with "maximal" stimuli, and are consistent with the notion that more than one impulse in one fiber is required to fire a synapse. They are not consistent with the notion of a one-to-one single fiber pathway from retina to cortex, inferred conventionally from data on sensory discrimination and degeneration experiments, but they are consistent with such a theory as that of "partially shifted overlapping," as proposed by Lorente de Nó (1934). Marshall and Talbot (1940) report similar findings.

The optic radiation also shows one type of variation in response to slowly repeated stimuli similar to that exhibited by the optic cortex, and in part responsible for the latter. With nerve stimuli at one second intervals, the second and following responses are equal, and definitely lower than the first, under the degree of anaesthesia employed. At five second intervals all responses are identical at the geniculate level (with further variation at the cortical level, see below). This phenomenon is more obvious when paired stimuli are repeated at 1 and 5 second intervals. With maximal paired stimuli at five second intervals, the second response of each pair is slightly but definitely higher than the first, with the appropriate interval up to 15 msec. between shocks of a pair. At one second intervals, the second and following pairs show a much decreased first response and a full-sized second response, which may be greater than an isolated response. That is, facilitation due to the conditioning response more than overcomes the depression due to the two responses occurring a second earlier. The same thing occurs with sub-maximal stimulation, with the added complication that weak stimuli may be complicated by depression of the optic nerve at the site of stimulation following the conditioning shock, presumably assignable to impaired circulation at the eye socket.

In responses recorded from two electrodes, one in the optic radiation and one in the cortex, similar phenomena indicating facilitation may be recognized in the cortex. Taking into account the amplitude of radiation spikes, facilitation of the second of a pair of responses may then appear in the cortical spikes following the radiation spike. (Fig. 2B.) The situation is more complicated for two reasons. First, the type of facilitation exhibited at the geniculate synapse also occurs at each cortical synapse, that is, the effect is cumulative during the course of the cortical sequence. One cannot say that facilitation at a given cortical synapse is any greater than that occurring at the geniculate level. Second, the amplitude of cortical responses varies independently of the size of the geniculate response. One factor involved in this variation is the phase of the spontaneous alpha rhythm at which the stimulus falls, the larger responses occurring when the stimulus is applied during the surface-positive phase (Bishop, 1933a). This effect is superposed on the

type of facilitation in the geniculate; at one second intervals, responses after the first are on the average lower than single or first responses, and the cumulative difference appears to be greater than in the case of the geniculate alone. At 3 to 5 second intervals, varying from animal to animal, recovery is complete (see also Marshall and Talbot, *l. c.*).

In animals exhibiting a marked variation in amplitude of cortical responses to stimuli at 1 per sec., a slight change of frequency may result in a fluctuation no longer random, and a slow waxing and waning occurs. This frequency is apparently one which is a simple multiple of the alpha rhythm, as can be demonstrated more positively in the rabbit (Bartley, 1936), where the alpha rhythm is usually more regular than in the cat. In the cat we have not been able to maintain such synchronism for more than a few coincidences or beats.

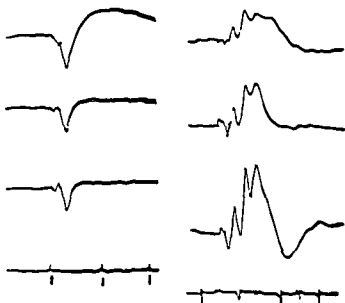
Aside from such effects, results of paired stimuli indicate that each successive synapse level of the cortex is susceptible to facilitation like that at the geniculate level, although we cannot correlate, in the more complicated records from that region, a facilitating effect with a slow potential of the character of the after-potential of nerve.

Homolateral responses to optic nerve stimulation. Of the three cell layers of the geniculate of the cat, degeneration experiments indicate that the middle layer receives the uncrossed optic nerve fibers (Minkowski, 1913). We have led from the geniculate of one side, while stimulating both optic nerves with single shocks, to determine whether any phenomena of interference or facilitation appeared that would suggest fusion of images at the geniculate level. (Fig. 3.) So far, no such evidence has been obtained. The relative magnitudes of response from stimuli to the two nerves have varied in different preparations from almost no homolateral response (10 or 15 per cent of the contralateral) to nearly equal responses (70 per cent). Responses from the cortex roughly parallel those from the geniculate in this respect in each preparation. In rabbits, the homolateral response of the cortex is always relatively smaller than in most cats; in 3 of 6 monkeys, in which records were taken from both optic areas, the responses from the two were approximately equal. (Fig. 3B.) Our results would indicate that cats are variable with respect to the homolateral projection of the retina on the cortex, and that facilitation between impulses from the two sides is relatively slight. Marshall and Talbot (*l. c.*) report facilitation at the cortical level but not at the geniculate.

Localized potentials. "Slow" potentials are also recorded from the cell regions of the geniculate. (Fig. 1 and 6.) No corresponding potentials are recorded from either tract or radiation, except that leads in the injured radiation, near a cut surface, may show after-potentials of considerable amplitude. Such injury potentials, which look like positive after-potentials, may appear immediately after a needle has been placed in the radiation, and decrease rapidly to disappear within a few minutes. (Fig. 3A.) They are probably a result of injury. The slow potentials of the normal tract or radiation are too low to be detected above the "noise" level of the region. In con-

trast to these, the slow potentials recorded from the vicinity of cells are specifically diphasic, follow the radiation spike rather than the tract spike, and the first phase has the same polarity as the spike which apparently initiates it. They are reminiscent of the slow processes observed by Gasser and Graham (1933) in the spinal cord, interpreted by them as the responses of intercalary neurones. The first or "negative" phase is also similar to the slow potential observed by Lorente de Nó (1939) in the oculomotor nucleus, interpreted by him as a slow depolarization of the spike negativity process of the cell body. We are inclined to the latter interpretation of our findings, especially since no intercalary neurones are present to which the process might be assigned. Further, the negative phase has the dimensions of the facilitation appearing after a first response.

FIG 3 Left column, 3 responses at 1 min intervals, the first immediately after inserting an electrode into the radiation. First, faint spike, tract wave, second, radiation, followed by a positive after-potential, the latter progressively decreasing. When the cortex is removed, leads from near the cut surface of the radiation show similar potentials that are more persistent. Time 5 msec. Right column, cortex responses, lateral gyrus surface to radiation, upper, homo lateral nerve stimulated, middle, contralateral, lower, both simultaneously with maximal shocks. The two upper records add up to the lower, showing no facilitation, except for the final wave below the base line. Time 5 msec intervals.



This potential might be interpreted as similar to the negative after-potential of nerve fibers, but is relatively much greater in amplitude. The slow potentials of two responses are summated, and the contribution of the second may be greater than that of the first. Therefore, we judge this slow potential to arise from cells or their dendrites in the geniculate nucleus, rather than from the fibers of tract or radiation, and to represent the sequella of the main response, rather than the immediate process of conduction which precedes it.*

* The records can obviously be interpreted either as those of a low wave (slow depolarization of the spike process), or as a process in the record (spike and slow process). The presence at the same region of different structures to which these processes might be assigned renders a separation by differential leads impossible here, and any theoretical interpretation is compromised by the assumptions one must make to account for potentials being led from such structures as cell bodies or dendrites at all. To account for such long-lasting potentials one must assume either slower conduction than is reasonable in this case (synapse time 0.5 msec), or localized differences in the degree of depolarization in different regions of the structure, persisting throughout the slow record. Assuming, for instance, that one end of the cell body, say the axon hillock, becomes more strongly negative than the rest, there seems to be

Repetitive responses of cerebral cortex. From the facts that the size of the cortical response varies, depending on the phase of the spontaneous alpha rhythm in which it occurs, and that as strychnine abolishes the spontaneous rhythm, it leaves the specific response higher than normal and less variable, we have inferred (Bishop, 1935) that optic nerve impulses at a frequency above that of the alpha rhythm would be relatively ineffective in exciting a given cortical pathway at more than about 6 per sec. (in the cat). This is a particularly significant factor since single volleys may set up not only the immediate cortical response, but also a train of alpha waves, which should then control the effectiveness of succeeding volleys. "Continuous" vision could then be assigned to successive activation of parallel pathways.

This interpretation requires modification in the light of the present work. In the first place, the facilitation described above acts to oppose the phasic depression connected with the alpha rhythm, if the frequency is sufficiently high. In the second place, anaesthesia not only decreases such facilitation, but greatly accentuates the depression of responses initiated during the surface-negative phases of the alpha waves. Thus the degree of anaesthesia employed in our previous experiments was a material factor in the results. The phasic depression is, however, not abolished in rabbits which have practically recovered from ether (Bishop and O'Leary, 1938), with wounds locally anaesthetized. Further, Heinbecker and Bartley (1940) have found the same cycle of depression and facilitation after a first volley to the saphenous nerve of cats, recording from the sensory-motor cortex under local anaesthesia and tetramethyl ammonium iodide. In these cases the second responses are depressed but not abolished, which must mean that some cortical elements are blocked, but not all of them. The sequence then appears to be as follows: depression in the relatively refractory phase of a first response; facilitation for a few thousandths of a second; depression during the surface-negative phase of the alpha wave set up by the first stimulus, and facilitation during the surface-positive phase, etc.

Records were taken from the optic cortex of cats with minimal anaesthesia, at various frequencies, by photographing a succession of lines across the oscillograph on a moving strip of paper. (Fig. 4.) Apparently the first volley sets up a complete cortical response, while successive volleys are lower, the later elements of each response being the more readily depressed. However, at about one-sixth second intervals there is an increase in amplitude of response, which at the frequency employed is grossly noticeable as a wider separation between successive lines of the record, followed by a crowding together. This results from the fact that each response serves as the base line

no objection to interpreting the spike as that of the axon itself, and assigning to the cell body only a low long-lasting potential. Such an inference would possess the virtue of accounting for repetitive responses to single or summated impulses on a relatively simple structural basis. It is compromised by the fact that such repetitive responses are normally found only where extreme structural complexity exists, and not, for instance, in the dorsal nucleus of the lateral geniculate. See Lorente de N6 (1939), Heinbecker (1932), and Renshaw, Forbes and Morrison (1940).

for the next, and summation or overlapping takes place. After a few cycles, all responses are approximately equal.

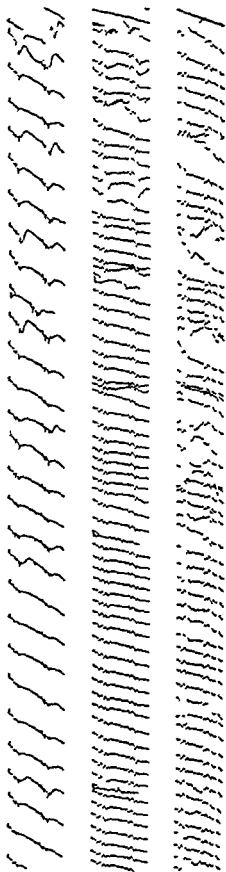
The events may be explained as follows. The first response sets up an alpha cycle, the depressive phase of which counteracts the facilitation that successive volleys might otherwise exhibit. The alpha cycles of the following volleys apparently do not develop, and the first alpha cycle controls the amplitudes of following responses, resulting in a second maximum one-sixth second after the start. During this maximum a second alpha cycle can be set up, but the fact that several shocks are delivered during this part of the cycle results in temporal staggering of alpha processes. For this reason the bursts at one-sixth second intervals do not persist, but soon average out, that is, the alpha responses in different elements become scattered and temporally dispersed to an average steady state. We have no conclusive evidence that alpha cycles are still present in each unit after they are no longer manifest in the summated record, but we infer their existence from the fact that if the cortex is stimulated only at one-sixth second intervals, or multiples of this, each response may be full height.

In normal vision this same chain of events should occur, but with much greater dispersion, due to the lack of initial synchronization of optic nerve impulses from the retina.

DESTINATION OF SIZE-GROUPS OF TRACT FIBERS

Histologically, properly directed sections through the optic tract, stained by Davenport's (1939) silver technique, show segregation of fibers according to size (Gudden, 1886), as indicated by potential records. The main bundle of optic tract fibers bends sharply medial to pass under the

FIG 4 Stimulation of optic nerve, leads from contralateral cortex, at rates of 40, 80 and 100 per sec, all supra-maximal. Successive sweeps photographed on moving paper strip, two responses to each sweep, 20, 40, and 50 sweeps per sec. The time can thus be estimated from the sweep frequency, as 50, 25, and 20 msec respectively for one sweep. Increases in amplitude of responses, and changed spacing in lines due to summation of successive responses occur at about one-sixth second intervals after the first stimulus, or with the frequency of the alpha rhythm. Further analysis in text.



geniculate proper at the lower apex of the ventral nucleus. A sheet of the remaining fibers leaves here to invest the ventral nucleus, especially its medial and posterior surfaces, and envelops the ventral and posterior surfaces of the dorsal nucleus, supplying fibers to these surfaces. Radiation fibers leave the lateral and anterior surfaces of the nucleus, which is arranged as a three-layered sheet bent into a S-shape (Rioch, 1929), the posterior limb of the S somewhat dorsal to the anterior. The posterior limb broadens out to form a hemispherical cap for the radiation; the anterior limb forms a similar cap for the division of the tract going to it. The medial curvature of the dorsal nucleus is continuous with the posterior portion of the pulvinar (Rioch, *l. c.*), and the tract to the colliculus runs below this, coming close to the surface posterior and medial to the groove between medial and lateral geniculate. (Fig. 5.)

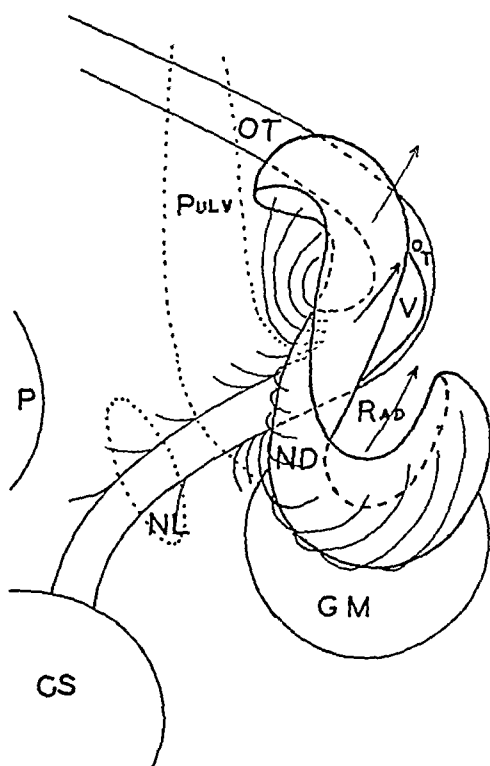


FIG. 5. A schematic diagram, not to scale, to indicate relations of optic tract to adjacent regions of the thalamus, as inferred from electrical records. Based on reconstructions from 20μ Nissl-stained sections, 0.36 mm. apart, normal cats; and a Marchi degeneration series after removal of one eye. Some fibers may also pass over the dorso-posterior surface of the geniculate and across the pulvinar to the pretectal region, as inferred by Barris, Ingram and Ranson. The whole region lying between pretectal area, superior colliculus and geniculate is traversed by optic tract fibers lying close to the surface. Synapse stations appear not to be confined to the specific nuclei usually assigned a visual function. Taking the dorsal nucleus as one limit of the distribution of optic tract fibers, and the superior colliculus as the other, the larger fibers distribute in general, but not exclusively, to the anterior regions and the smaller to the posterior, as indicated by post-ganglionic responses to shocks of different strength which are shown to activate fibers of different sizes. OT, optic tract. ND, nucleus dorsalis of lateral geniculate. V, nucleus ventralis. GM, medial geniculate. Rad., optic radiation. Pulv., pulvinar. NL, nucleus lenticiformis mesencephali. CS, superior colliculus. P, pretectal area.

The division of the tract passing to the geniculate, and entering its cell layers, contains most of the largest fibers of the nerve, and some medium sized. Passing with this bundle, however, are some of the smallest fibers of the nerve. We have been able to recognize no responses from these small fibers in the geniculate, nor to detect any secondary effect of their stimulation in the optic radiation or cortex, which may indicate only that our technique is not sufficiently discriminating.† The remaining tract contains me-

† A tract second spike and its post-ganglionic response can be recorded from the re-

dium sized fibers and many more fine fibers than appear in the tract to the dorsal nucleus. It is not clear which, or how many, of these fine fibers are the fine branches of coarser geniculate fibers recognized by Barris, Ingram and Ranson (1935), but most of those to the colliculus, and many of those to the dorsal nucleus, obviously come from the optic tract and nerve, and as suggested by a corresponding slow potential in the frog optic, presumably arise in the retina.

Records from various regions of the optic tract distribution (Fig. 6.) indicate that in general the smaller fibers pass further posterior, toward the colliculus, before they synapse than the medium-sized, while the larger fibers pass a shorter distance, to the geniculate. Between these two bodies lie various structures which have been inferred to receive optic tract fibers, such as pulvinar, nucleus lentiformis mesencephali, and pretectal area. Employing as criterion not only a record of the second tract wave, but a post-ganglionic wave which follows this, and which does not follow weaker stimuli that excite the first tract wave, we have traced such fibers to some of these structures and also to the marginal territory along the tract bordering the medial geniculate nucleus, and to the territory just medial and anterior to the dorsal nucleus of the lateral geniculate. These post-ganglionic responses are very well localized, and movement of the critical electrode half a millimeter in the vertical plane may determine their presence or virtual absence in the record. Pending further work now in progress, at present the following scheme of optic tract fiber distribution emerges.

The main bundle, from the region ventral to the ventral nucleus of the lateral geniculate, to the superior colliculus, loses fibers continuously, the contribution to the dorsal nucleus being only the start of this process. Seen in cross section beyond the dorsal nucleus, the tract appears as roughly V-shaped, but with a fin extending medially along the surface of the thalamus, toward the pretectal region. Cells lying along and below the fiber tract appear to be synapse stations for tract fibers, as well as do the more discrete anatomical nuclei usually assigned to the visual system. In general, the larger fibers distribute over the anterior end of this fan, the smaller over the posterior end, although some fibers of all sizes are present at any level.

The significant feature of this distribution appears to be that separate groups of fibers, from the retina on, as distinguished by threshold for electrical stimulation and by conduction rate, supply these various regions. This may be contrasted with the usual interpretation, that in the propagation of sensory impulses toward the brain, one collateral of a given fiber synapses in the thalamus with a neurone carrying the impulse to the cortex, another collateral of the same fiber passing to secondary nuclei of thalamic or reflex

gion where the dorsal nucleus of the geniculate passes over into the pulvinar, but it is difficult to say whether this is, properly speaking, geniculate territory. This forms no exception to statement made previously that only the first spike process appears to activate the cortex; for while the post-ganglionic responses can be detected locally, no increase is seen in cortical response when they appear. Work tracing such responses further is in progress, together with degenerative experiments after electrolytic lesions in critical areas.

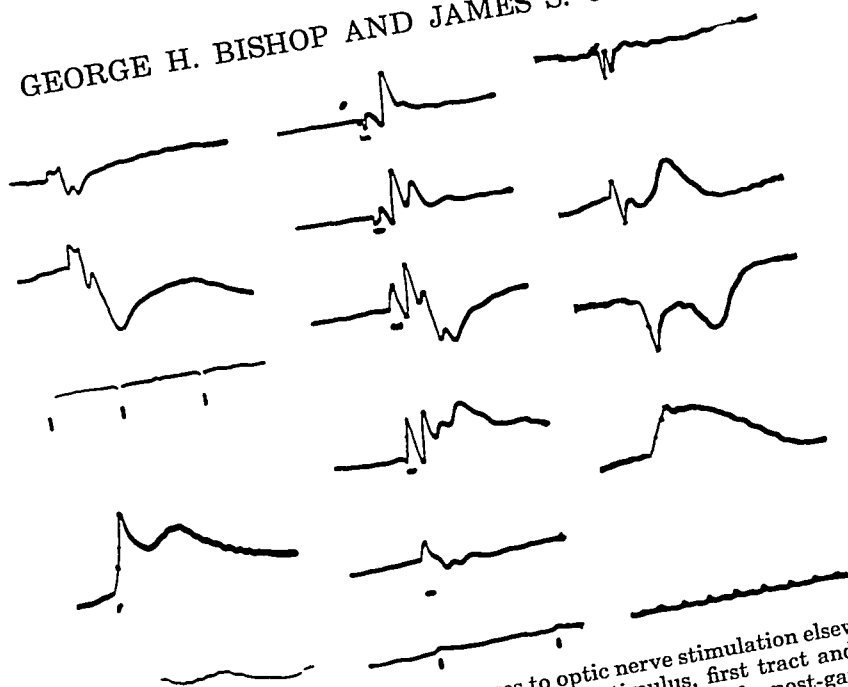


FIG. 6. Records of post-ganglionic responses to optic nerve stimulation elsewhere than from the dorsal nucleus of the geniculate. Left, weak stimulus, first tract and radiation spikes, and below, stronger stimulus, second tract wave and start of a post-ganglionic response. One needle in margin of tract adjacent to medial geniculate, where most of large fibers have left tract to enter lateral geniculate; one needle in hippocampus dorsal and medial. Time 5 msec. Below, record on slower time line of same response to show late response (amplifier connections reversed, record inverted from above). Bottom, same, needle withdrawn 0.5 mm. with marked decrease in amplitude, needle now in groove behind parietal cortex, one in ventral nucleus of lateral geniculate. Weak stimulus, $\times 1.5$, second tract spike becomes diphasic, and trace of radiation spike. Next, stronger stimulus, $\times 4$, second tract spike becomes diphasic, and trace of radiation spike. Third record, stimulus $\times 4$, second tract spike slightly half maximal, monophasic. The second spike is followed by a post-synaptic response, and although the spike occupies nearly the same time position as the second spike in the record above, causes a downward deflection, as if the two groups of fibers passed on opposite sides of the electrode. Fourth record, leads one in groove just posterior and medial to ventral nucleus of lateral geniculate, and one deep in brain stem under pre-ectal region. First tract spike unchanged, second spike no longer diphasic, post-ganglionic spike reversed. The difference between the last two records is due to the fact that the tissue responsible for the post-ganglionic response is between leads in the first case, and outside, or surrounding one lead in the second. This locus corresponds to the ventral nucleus or regions just behind and medial to it. Lower record, leads are the "indifferent" electrodes of two records above, to indicate that the responses do not come from the vicinity of either. The first deflection, as in records above, is stimulus artifact, and a trace of distant responses is picked up as the arm leads will record the electrocardiogram from the superior colliculus; short DC stimulus, strength for first, maximal for two tract spikes, for second, $6 \times$ this, just maximal for late response reversed; surface negative, deep positive. Note that just preceding the large wave is a smaller one which does not reverse, is recorded as from the tract electrode negative, and appears only at a stimulus strength producing the large post-ganglionic response. Fourth record, slow potential from dorsal nucleus of geniculate in same cat, compare Fig. 1 and Fig. 7. Time 5 msec. The various slopes assumed by the base lines of these and previous figures are due to spontaneous waves of the order of frequency of the cortex alpha rhythm.

function. To be sure, many optic tract fibers do divide, with only one of the branches passing to the lateral geniculate, as Barris, Ingram and Ranson have reported. Our experiments suggest that the characteristic activity of each fiber group is manifest at one locus; that is, that the majority of the fibers which activate the cortex do not activate the superior colliculus, and vice versa.

This situation indicates that in the phylogeny of the visual system, the outgrowth of the geniculate-cortex complex from the thalamic mass may have involved the functional separation of two groups of cells *and their connections* back to the retina, instead of two groups with fiber connections in common, and that the group characterized by the fibers of most rapid con-



FIG. 7. Parasagittal section through lateral and medial geniculate bodies, electrolysis locus of needle giving slow wave of last record, Fig. 6, in cell layers of dorsal nucleus of lateral geniculate.

duction and largest size have come to serve the higher centers. Experiments are now under way to test whether retinal stimulation by light can be so manipulated as to stimulate one group of fibers or another, or whether, perhaps, the same sense organ distributes impulses in the retina to fibers of different groups.

DISCUSSION

Records from any region in the thalamus are written upon the spontaneous slow rhythms present as a base line. In view of the fact that the cortical response to nerve stimulation appears to vary with the phase of the spontaneous rhythm of the cortex, we have recorded thalamic and particularly dorsal geniculate spontaneous rhythms to see whether they were in phasic relationship with the rhythm of the optic cortex. So far this has never been the case, in which we confirm Dubner and Gerard (1939). Moreover, the optic radiation responses to electrical stimulation of the nerve vary neither with

the geniculate rhythm nor with that of the cortex, from which we must conclude that the influence assignable to the alpha rhythm is exerted at the cortical level. We have thus been unable so far to find evidence, in the geniculate, of fibers that would complete a circuit from geniculate to cortex and return, as previously inferred from the circumstance that cutting the radiation either just below the cortex, or even just above the geniculate, abolishes the alpha rhythm. If such fibers exist, they must pass from some structure so near the dorsal nucleus that severing the radiation close to the latter also severs the pathway in question. That is, we should look for a pathway from thalamus to cortex which mixes anatomically with the radiation from the dorsal nucleus, but which converges with it functionally only at cortical synapses; and the origin of this pathway may be expected to be a nucleus reached by corticofugal fibers, and exhibiting a rhythm in phase with that of the cortex. We are the more persuaded that such a pathway will be found to exist since Dusser de Barenne and McCullough (1938) have demonstrated a similar indirect return circuit through an "accessory" thalamic region in the case of the sensorimotor cortex by means of local strychninization.

Allowing for conduction and synapse time in pathways to various parts of the optic mechanism, it appears that of two impulses started in parallel fibers of the optic tract at the retina, the one passing to the cortex will result in a return message to the colliculus, for instance, before the other has reached the colliculus directly. It would thus be possible on physiological grounds for an animal to recognize a sensory stimulus in a shorter time than it could respond to it reflexly; or, looked at otherwise, there would be time for a message from the cortex to reach reflex centers for regulation of the response before the reflex centers were activated directly by the same stimulus. Not only is there time for such a process, but such impulses do arrive at the colliculus from the cortex (Bishop and O'Leary, 1938) and also at the pretectal region. We have not traced them to the ultimate response.

In evaluating the part played by facilitation at the various synapses along the optic pathway, we are inclined to distinguish between the two types observed, the one resulting from a conditioning shock a few milliseconds previous, the other associated with the alpha wave process. The former is similar to that which occurs in the cervical sympathetic ganglion, cord, oculomotor nucleus, etc., and probably will be found at any synapse. The latter may be more characteristic of "spontaneously" active regions. In evaluating the part played by either (or by the interaction of both) in the organization of the response of the cortex to a given sensory stimulus, it will presumably be necessary to go beyond the effect of single or repeated volleys induced by peripheral nerve stimulation, and take into account the pattern of response induced in nerve fibers by activation of sense organs. The problem presents itself in the terms: what is the effect of a given *pattern* of peripheral nerve excitation upon a given *pattern* of cortical receptivity, whether the latter is a function of spontaneous activity or of previous stimulation. In this connection, the statement will bear repetition that the state of cortical

receptivity for extrinsic stimulation seems to be bound up with the activity represented by spontaneous cortical rhythms.

SUMMARY

1. Four groups of fibers in the optic nerve produce four potential waves after conduction. The geniculate and cortex are activated chiefly by the first group of fastest conduction, with one radiation spike only following each nerve volley. Paired shocks exciting these fibers in the optic nerve, even when maximal, show facilitation of the second responses at intervals of 2 to 15 or more msec. The same applies to successive synapses traversed in the cortex, the results being cumulative.

2. The homolateral response of the optic cortex in cats varies from 15 to 70 per cent of the contralateral. No facilitation can be detected at the geniculate level by simultaneous or successive stimulation of the two optic nerves, and little, if any, at the cortical level.

3. Slow potentials of the order of after-potentials in their time relations can sometimes be demonstrated at electrodes thrust into the cell layers of the geniculate. They seem to be non-conducted, and are interpreted tentatively as slow decay of cell body or dendritic excitations.

4. The cortical activity shows a depression followed by facilitation having a phasic relationship to the alpha rhythm, after a single volley in the optic nerve. The short-period facilitation occurring at geniculate and cortex counteracts this depressive phase so that with light anaesthesia responses of the cortex will follow up to at least 100 per sec. Fluctuations of amplitude in these responses still follow the alpha wave set up by the first stimulus of the train, until temporal dispersion of alpha processes occurs.

5. Fibers from the optic tract spread in a thin surface sheet over much of the area bounded by the medial and lateral geniculates, the pretectal area, and the superior colliculus. From regions not usually assigned optic function, post-ganglionic responses can be recorded. In general, the larger fibers with faster conduction rate and lower threshold synapse in the anterior regions of the optic tract distribution, the smaller in the posterior regions.

6. The chief conclusions we can draw from these findings lead to further hypotheses to be tested rather than to an explanation of visual function.

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INTEGRATION OF LOCOMOTOR BEHAVIOR PATTERNS OF THE HAGFISH

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INTRODUCTION

THE California Hagfish, *Polistotrema stouti*, is an ideal subject for the study of simple locomotor behavior patterns. Lacking paired appendages, and relatively unspecialized in nervous makeup, it possesses but one means of progression. This consists of swimming with a sinuous or snakelike undulating action. Progression is normally forward, but proper stimulation will result in a reversal of the pattern and backward swimming. For an account of the mechanics of such progression see Gray (1933).

The undulating behavior, though illustrated well in the hagfish, is not peculiar to it. All fish and aquatic amphibians swim with this method or a derivative of it. Gray (1936) has suggested that this type of locomotion underlies the stepping reflexes of mammals. With this view I am in complete accord. If the stepping reflex is specialization of the undulating pattern, one may say that every species of chordate (except the degenerate adult *Ascidian*) employs the same fundamental plan of locomotion. Thus particular weight must be given to the opinion of de Lange (1936) that the mesoderm segmentation so characteristic of the phylum, as well as the notochord which gives it the name, is no more or less than adaptation for this locomotor behavior.

Considerable error has crept into the older literature concerning the nature of these undulatory waves and the mechanisms by which they move. There is seemingly a natural tendency to link the rate of transmission of the waves with the speed of the nervous impulse over long pathways. For a clear understanding, it must be borne in mind that the actual wave of contraction is a product of local integration and hence the speed is variable. As will be shown below, the waves may stop their motion or go into reverse. Thus they represent not the passage of impulses in descending tracts of the spinal cord touching off the final common pathway, but a wave of excitation of the internuncial as well as the motor neurons.

METHODS

The hagfish were caught in Monterey Bay and kept in large tanks until used. * Ether shaken into seawater served as anesthetic. Operations on the spinal cord were especially easy because no bony or cartilaginous tissue surrounds the central nervous system. The animals are so hardy that extensive operative trauma will not kill them. Spinal shock is minimal. No recording devices were set up, visual impressions of activity were used in interpretation. Because of the possibility of regeneration of nerve fibers all observations were

* I wish to thank the director of the Hopkins Marine Station, Dr. W. K. Fisher, for placing material and equipment at my disposal during the summer quarter of 1939.

made within three days of operation. Stimulation was effected with a Harvard inductorium set to deliver the weakest effective current.

EXPERIMENTAL RESULTS

In the course of the experiments, the brain was pithed in a number of instances. Spinal shock was minimal and characteristics of the spinal hagfish were to be seen as soon as the ether had worn off. Because cephalization is so slight in these animals few deficiencies were evident. The spinal preparations lost the equilibratory reflexes and rolled over when swimming. They became inactive and lay on the floor of the tank for long periods of time. Sufficient stimulation, however, would set them off on long swims during which their locomotion was essentially normal.

When a hagfish is subjected to simple spinal cord section, the anterior and posterior parts of the body acquire a large measure of independence in their behavior. Stroking the tail in the intact animal causes waves of undulation to start immediately behind the head and continue in the normal or backward direction. If, however, spinal section is performed, a new site functions in the initiation, since the waves begin just posterior to the section. This occurs no matter where the cord is divided—even the base of the short tail can serve as site of origin for waves of normal direction. Reverse waves, progressing anteriorly, are the usual result of stroking the gill region. Normally these take origin near or at the tip of the tail. Where the spinal cord is divided, the site of origin of these waves shifts to the part of the cord just anterior to the operation. This occurs when the section is situated anywhere from the tail to just behind the head. The conclusion which must be drawn is that while in intact individuals normal waves generally are initiated near the head end, and reverse undulations near the tail, nearly all segments of the cord possess the ability to initiate waves of both directions.

An experiment was performed which illustrates the nature of the undulating locomotor waves. Hagfish no. 21 was etherized to the point that scarcely any reflexes could be elicited. Then a pair of dull scissors was inserted in the back behind the head and the cord severed by repeated chewing movements. Violent reverse undulations set in which continued for several minutes. As these weakened it was observed that occasionally waves would start near the tail and travel to the anterior part of the body and then slow down. Some would change direction and proceed a short way backwards. These would seesaw back and forth several times. The course of the waves could be governed by stimulation on the surface of the specimen. When they slowed down near the middle of the body a pinch on the tail would start them going posteriorly. Before they reached the tail their direction would change if the gills were stroked. This type of manipulation was possible for several minutes, after which a state of fatigue set in and no activity could be elicited. As in the normal animal, it took a much lighter stimulus on the tail than on the gill region to be effective. It is important to note that the speed and direction of the waves are variable and, though our terminology becomes paradoxical, the waves may be stationary for short periods. What

was observed in this experimental animal was apparently the mechanism which governs much of the activity of the normal one. Stimulation of the gill region causes reverse swimming; stimulation over most of the rest of the body and particularly on the sensitive tail induces forward locomotion. Where both types of stimulation are given, the result depends on the relative strength of the stimuli.

That stimulation of the anterior end of the cord causes the initiation of waves near the tail and vice versa indicates that at the beginning of the activity the cord is functioning as a whole. Striking proof that the undulations continue to be integrated by the entire intact portion of the cord is shown by the fact that the wave-length, from crest to crest, of the bendings are proportional to the length of the integrating segment. In the pithed hagfish this distance may be ten or twelve inches. As the cord is shortened by successive caudal sections, the waves become shorter. Where only the cord posterior to the anus is left, the tail bends into very sharp curves, less than an inch long. Thorner (1932) has observed this phenomenon in snakes. Such findings are adequate proof that locomotor activity is the product of the whole spinal cord and not just those parts innervating the active muscles.

There is much interest in the matter of the coordination between the head and tail ends of an animal with a spinal section. This is the subject of two papers by Ten Cate and Ten Cate-Kasejewa (1933) who, through experiments on the dogfish, confirm the theory of Hooker and Nicholas (1930) that the coordination between front and hind segments of the body is effected by the overlap of proprioceptive sensibility and the consequent reformation of the waves behind the site of section through stretch reflexes. Ingeniously they tested this theory by removing parts of the cord several segments long and by sectioning nerve roots above and below simple sections. From these they concluded that a denervated region of five segments in length is such a barrier to the transmission of undulatory waves that only after locomotion has proceeded in the experimental animal for some time does the rear part show coordination with the fore. Gray and Sand (1936) are unable to support Ten Cate's contention that the rear part of a dogfish can coordinate its activity with the portion anterior to spinal section. They suggest that error may be introduced into Ten Cate's experiments through his reliance on visual impressions of activity. In the experiments which I carried out on the hagfish, visual impressions were also the only method of observation used. Yet the distinct impression was gained that coordination was sometimes possible over complete division of the cord. Several experiments of this type were performed, but in one in particular three sections were made—one immediately behind the head, one at the half way mark, and one behind the anus at the base of the tail. The hagfish survived well and showed symptoms which may help explain the disparity between the conclusions of Ten Cate and of Gray and Sand. Following the operations, it was inactive and lay on its side in the tank. Appropriate stimulation would cause independent swimming movements in any of the four segments, but

locomotion did not result from such activity. When the specimen was persistently handled and subjected to considerable stimulation, the parts would be brought into coordination and it would swim across the tank nearly as well as a normal animal. This successful activity always followed a short period of ineffectual and uncoordinated struggling. My impression was that strong stimulation led to coordinated movements while weak stimulation produced ineffectual and unrelated activity of the parts. This interpretation is supported by the action of animals with spinal hemisections to be described below. It may be that the dogfish which Ten Cate observed had been roughly handled and otherwise subjected to strong stimulation and that those of Gray and Sand, on the other hand, were insufficiently roused. As to the method of coordination between the isolated segments, my experiments shed no light.

In one specimen removal of a section of the cord equal in length to four or five myomeres resulted in a permanent incoordination between the two parts. It must be remembered in comparing this with Ten Cate's more successful operations on the dogfish that because of the absence of a spinal column the hagfish is a much more flexible animal and consequently mechanical transmission would be more difficult.

Hemisection experiments illuminate this matter from another angle. If the spinal cord is cut half way across in a spinal or intact hagfish, striking symptoms are not shown. There is more than usual sluggishness, and the coiled resting posture typical of intact animals is altered. Reverse undulation disappears behind the site of the operation. But forward locomotion is normal. It is not until one makes paired hemisections that striking deficiencies show up. When the two hemisections (on opposite sides of the cord) are situated about 3 cm. apart the symptoms resemble those of a simple complete section in some particulars. In an experiment of this type the protocol showed the following points. Within a few hours of the operation no signs of transmission across the two lesions could be observed. When the head was held, reversing began in the anterior section of the body, the posterior remained passive. When the tail was pinched normal undulations began near the operated segments to proceed toward the tail. Repeated stimulation would sometimes cause the head end to reverse, the tail end to exhibit normal undulation. But the next day there was transmission across the injured region. When waves which started at the head neared the operated segments, they would noticeably slow down with the effect that they became closer to one another. When the head was seized, reverse waves commenced near the paired lesions and only the head end reversed. Continued holding caused the pattern to break through the area of the lesions and the reverse waves began, as in the normal animal, at the tail.

More interesting are paired hemisections when separated by a third or a half of the length of the body. One animal on which this experiment was carried out emerged from the anesthetic, which had been light, in an extremely active state with the highly exaggerated reversings which form the

well-known figure-of-eight writhing of this species. After the animal had quieted down it was noted that, while stroking the snout would cause undulations of the part anterior to the first hemisection, holding the animal by the gills would cause the whole body to reverse in a coordinated manner. The next day it showed a slow, rhythmic, spontaneous undulation of the anterior segment of the body. Waves of rather small but equal intensity passed alternately down each side. But those on the side of the first hemisection, the right side, were stopped at that point, while the left-sided waves proceeded to the second hemisection, where they too were halted. In the middle part of the body, thus, left-sided waves were not accompanied by the usual right-sided ones. The contractions were opposed only by the elasticity of the notochord and associated structures so that between the curvings to the left, the body straightened out, but did not curve to the right. Still, in this animal, a strong pinch on the tail caused rapid and normal locomotion with all segments of the body working.

The unexpected results of the two experiments just described have led me to view the effects of hemisection of the cord as that of a resistance to transmission of locomotor waves. In the first example, the waves slowed down as they approached the lesions. And the reversing, which was confined to the anterior part of the body if the stimulation was light, would break through and involve the whole animal with harsh handling. The latter experiment showed the dissociating effects of the hemisections—right-sided waves could be filtered out, as it were, allowing their counterparts on the left to proceed further down the animal. Yet again, strong stimulation tended to break through and result in normal behavior.

It would obviously be unjustified, on the basis of these gross experiments, to attempt to erect an elaborate explanatory hypothesis, and the comparison, made above, of hemisections to points of resistance must be considered descriptive and not any attempt at theory.

So remarkable did the dissociation of the right and left halves of the behavior pattern by double hemisection seem that direct stimulation of the spinal cord by means of a faradic current was tried. The results check the first observations. When the spinal cord of a hagfish was exposed it was found that placing the electrodes on the right side of the cord caused waves to pass forwards and backwards from the point of stimulation on the right side only. When the electrodes were moved to the left side, the waves affected that side only. Yet it should not be assumed that this effect was due to the stimulation of long tracts, because the progress of the waves was too slow to be due to simple transmission over single or multiple neuron pathways. There is no reason to think that these one-sided waves were not integrated as are the normal ones and thus capable, under proper conditions, of changing both their speed and direction.

The dissociation of the right and left elements of the locomotor behavior pattern focuses attention on the nature of the waves themselves. That such a finding does not mean that the two sides are unrelated in their activity is

indicated by the fact that no manipulation caused waves on one side to pass by those on the other. The left-sided waves were always oriented with respect to those on the right in such a way that they were alternate in placement and travelling in the same direction. The possibility yet remains that an integral part of each wave of contraction is a contralateral wave of inhibition or relaxation.

SUMMARY

1. The California Hagfish, *Polistotrema stouti*, was subjected to operational procedures designed to illustrate the nature of the locomotor behavior patterns. Visual impressions of the deficiencies produced were used for interpreting the results.

2. Simple cord section produces a disintegration of the total behavior pattern which can be reestablished under strong external stimulation.

3. That the behavior studied is a total pattern is indicated by the fact that isolation of a segment of the cord causes an immediate formation of a new site of initiation of the waves and also in that the wave length of the undulations integrated by isolated segments varies with the length of the segments.

4. The undulatory waves represent moving sites of nervous integration and their speed and direction may be experimentally altered.

5. Stimulation of the posterior end of the body and particularly of the tail results in the usual head-to-tail undulations. Stimulation of the gill region initiates waves of reverse direction.

6. Single hemisections of the cord do not incapacitate the animal for forward locomotion, though backward swimming becomes impossible. Paired contralateral hemisections act as complete sections except that with strong stimulation, the pattern was more easily restored to normal.

7. It is possible, with properly placed hemisections, to dissociate the undulatory pattern into right and left-sided halves. Direct faradic stimulation of the sides of the cord also produced this fractionation.

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EFFECTS OF HEATING HYPOTHALAMUS OF DOGS BY DIATHERMY*

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THE hypothalamus functions in physiological temperature regulation in two ways. It acts as an integrating and coordinating center for nerve impulses from thermal peripheral receptors (peripheral or reflex temperature regulation) and functions as a "thermostat" since it can activate the heat loss or heat conservation mechanisms when stimulated by temperature changes of the blood (central temperature regulation). This dual function of the central nervous system first described by Richet (1884) has been the subject of a number of investigations of recent years (Fulton, 1938). The exact hypothalamic areas which must be intact for temperature control have been mapped by means of the Horsley-Clark lesion method by Teague and Ranson (1936), Ranson, Fisher and Ingram (1937), and Clark, Magoun and Ranson (1939). The regions of the hypothalamus which are responsive to warmth stimuli have been mapped by Magoun, Harrison, Brobeck, and Ranson (1938) using bipolar Horsley-Clark needles between which a high-frequency diathermy current was passed causing heat without electrical stimulation.

HISTORY AND METHODS

In order to determine to what extent physiological temperature regulation is central or peripheral it is necessary to produce central temperature changes without peripheral changes and vice versa. This is not possible with the usual methods of heating or cooling in which an animal is exposed to a warm or cool environment. With such a procedure *both* peripheral and central temperatures are raised or lowered simultaneously. To investigate central temperature regulation uninfluenced by disturbing peripheral changes, it is necessary to raise or lower the hypothalamic temperature without changing peripheral temperatures. This experiment requires local heating of the brain with a constant skin temperature. Attempts have been made in the past to produce local heating of the brain by cooling or warming the blood passing to, and in some cases from, the brain (Kahn, 1904; Moorhouse, 1911; Barbour and Jelsma, 1931). Barbour (1912) introduced the "warmestich" method in which a heated or cooled metallic cannula was inserted into various parts of the brain there by producing local heating. These experiments, necessarily acute, have been complicated by anesthesia, surgical procedures, the difficulty in separating blood of the face from that of the brain, and the heating of peripheral structures by venous blood from the brain. Cloetta and Waser (1913) used large external diathermy electrodes outside the skin of the head but this type of heating would heat the skin of the head as well as the brain. The method of Magoun, Harrison, Brobeck, and Ranson (1938) is an improvement over the earlier procedures since excellent localization of heat is produced but anesthesia and surgical trauma caused by insertion of needles through thalamic and hypothalamic structures are possible complicating factors.

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To produce local hypothalamic heating of a normal unanesthetized animal without surgical trauma of the brain small gold diathermy electrodes were placed on the anterior or posterior hypothalami of dogs. Small wires attached to the gold electrode were brought to the skin surface and at least one month allowed for recovery. Quantitative tests for temperature regulatory function such as vasoconstriction, vasodilation, shivering, and panting were made and the responses found to be normal. The brain was then heated by local diathermy and the temperature regulatory responses measured. An anatomical examination of the hypothalamus after completion of the investigations showed the brain to be normal in every respect.

Operative procedure. Nine dogs were carefully chosen for their ability to cooperate in temperature regulation tests not requiring anesthesia. The operation consisted of removal of a bone flap from the temporal region and placement of small gold electrodes with attached wires on either the anterior or posterior hypothalamic areas. The anterior electrode was placed between the optic tracts and extended over the chiasma and to the pituitary stalk. The posterior electrode was placed between the pituitary stalk and the mammillary bodies. The electrodes were approximately oval in shape with diameters of 3 and 6 mm. Two wires, one manganin and the other copper, were insulated from one another and attached to the electrode. The two wires were wound with silk, covered with several thin layers of bakelite enamel and baked in an oven at 100°C. to harden the enamel. Such insulation remains unchanged in all experiments and exhibited no deterioration even after 6-8 months in situ. The electrode wires were fastened to the bony edge of the trephined opening, the free ends of the wires were brought to a subcutaneous position near the mid-line of the superior surface of the skull and the skin flaps sewed over them. Not less than one month after the operation and when temperature responses were normal, as shown by tests, the free ends of the wires were located by x-ray and brought to the exterior through small openings in the skin. In two experiments small gold electrodes were placed in the temporal lobe and in one case a small free thermocouple was placed along the side of the optic tract about 2 mm. from the edge of the electrode. There were no deaths resulting directly from the operative procedure or immediate infection. In one instance a "pus pocket" developed and followed the electrode wires to the brain and extended as a circumscribed area over the pons resulting in the death of the animal 6 months after the operation. There were no signs of infection in the other animals. In the 2 animals described in detail later there was no evidence of damage to the hypothalamus.

EXPERIMENTAL

Tests for normality of temperature regulation. Following the operation the dogs were allowed one month for recovery after which tests were made to determine whether or not the temperature regulatory functions were normal. The physiological mechanisms tested were (i) shivering, (ii) peripheral vasoconstriction, and (iii) peripheral vasodilation and panting. The vasomotor and shivering tests with values for normal responses are described in a previous paper (Hemingway, 1940). Briefly, they consisted of allowing the trained dogs to rest on a shivering recorder in a cool but not uncomfortable room with controlled air velocity, temperature, and humidity. Thermocouples were attached to the skin of the ears, thorax, foreleg, and beneath surface diathermy electrodes of which one was placed beneath the recumbent dog and the other on the upper surface of the thigh. Body temperature was measured by a rectal thermocouple. Temperatures were measured every three minutes during the experimental period. After peripheral vasoconstriction (measured by ear temperature) and shivering had occurred and a steady state of temperatures had been reached, the dogs were heated by diathermy with a heat dosage rate equal to the basal metabolic rate. The heating was continued until peripheral vasodilation, as denoted by a sudden rise in ear

temperature, had occurred. Temperatures were plotted against time on a graph and the threshold peripheral and body temperatures were determined at which shivering and peripheral vasoconstriction commenced and ceased. Similar tests were made for panting in a warmer controlled environment and using diathermy as a means of a measured heat stimulus. The temperature regulatory responses were normal when the threshold temperatures fell within the normal ranges.

Estimation of local brain temperatures. An exact determination of local brain temperature is not possible by the method used for heating. This is due to the fact that the major portion of the heat production occurs in the tissue in immediate contact with the electrode and a steep temperature gradient occurs as the distance from the electrode increases. A free thermocouple 2 mm. distant from the brain electrode indicated body temperature under all conditions of heating. Also the brain electrode was enclosed in a connective tissue sheath which had grown around the electrode in the post-operative convalescent period. Nevertheless, the electrode temperature could be measured with an accuracy of $0.2^{\circ}\text{C}.$ by means of the attached thermocouple and the heating conditions could be reproduced at any time by adjusting the diathermy current. For each experiment a graph was made of electrode temperature for various heating currents. After this calibration any desired temperature could be obtained by adjusting the heating current.

Electrode temperatures for the various heating currents were as follows:

mA	Degrees C. above rectal
50	0 5°
70	1 0°
100	2 5°
150	4 0°
200	8 0°

The actual brain temperatures were much lower than these upper limiting temperatures due to a connective tissue sheath around the electrode and a steep temperature gradient from the electrode. In the case of anterior hypothalamic heating the optic chiasma was interposed between electrode and thermosensitive hypothalamic tissue.

Local heating of brain. When the temperature regulatory tests had shown that the animals had normal responses to heat and cold the local brain heating experiments were started. In these experiments the dogs were placed on a shivering recorder in an air-conditioned room of $22.0 \pm 0.5^{\circ}\text{C}.$, relative humidity 50 ± 5 per cent and air velocity 25–40 ft. per min. After resting 30 to 90 min., peripheral vasoconstriction first occurred as indicated by a rapid drop of ear temperature and was soon followed by shivering. The mechanical shivering recorder has been described in a previous paper and five degrees of shivering intensity are recognized in an arbitrary classifica-

tion. A shivering intensity 5 represents maximum intensity, 0 represents no shivering, and intermediate degrees of intensity are represented by No. 1-4. The dog lay on his side on a metallic electrode which made electrical contact with his shaved lower side. This lower electrode is the indifferent diathermy electrode, and the skin in contact with this large electrode never undergoes a temperature change when the brain is heated. Skin and rectal temperatures were obtained throughout with thermocouples. When shivering had become sufficiently intense to maintain a steady state of body temperature the brain was heated by passing a high frequency (diathermy) current between the small (active) brain electrode and the indifferent electrode. The currents varied from 40 to 200 mA and were continued for 2 to 20 min. with heating rates of 1 to 5 w. Respiration rate was measured by a chest cuff and tambour. In studying panting similar tests were made except that the room temperature in some experiments was raised to 26°C.

RESULTS

Anatomical. Of the 9 operated dogs two will be described in some detail. These two were the most successful of the series, being free from accidents

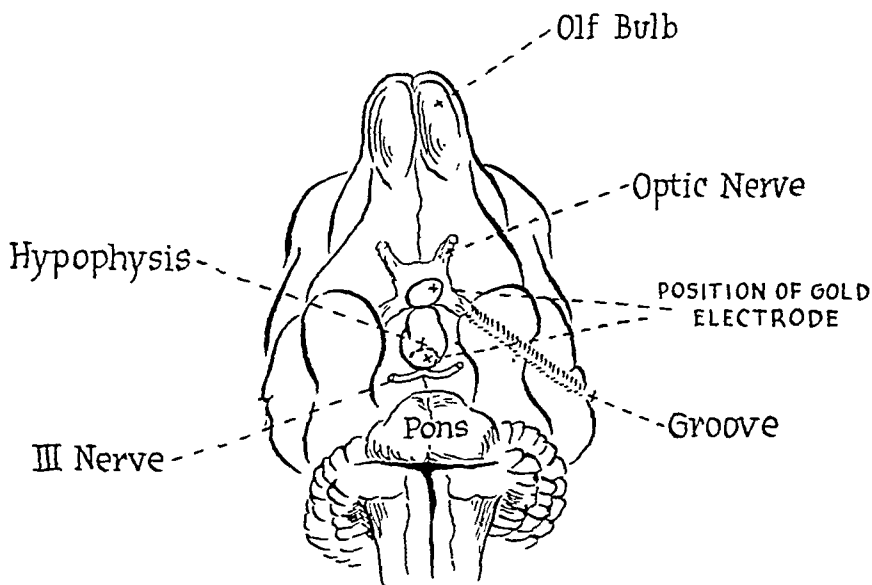


FIG. 1. The position of the hypothalamic surface electrodes is shown. Only one hypothalamic electrode was used on each dog.

and possible errors. The results obtained from the other 7 were confirmatory of the results from the two described. Of these two one had an electrode on the anterior hypothalamus over the optic chiasma and extending to the tuber cinereum (see Fig. 1). The other animal had an electrode on the posterior hypothalamus extending from the mammillary bodies to the pitui-

tary stalk (see Fig. 2B and C). The exact location of the posterior electrodes on the brain is shown in the x-rays of Fig. 2, one electrode being used on each dog. An attempt was made to place both an anterior and a posterior



FIG. 2 A. The position of the posterior electrode is shown by an x-ray exposed from above the head. B. A side view of the same animal.

electrode on the same brain but the dog lived only one week and, since the purpose of the experiments was to have the animals as normal as possible it was considered advisable to reduce hypothalamic manipulation to a minimum and use only one hypothalamic electrode per animal.

A careful histological examination of the hypothalamus beneath the electrodes was made and this region was found to be normal. There was a slight amount of temporal lobe erosion where the electrode wire was in a subdural position but otherwise the brain was uninjured.

Tests for normality of temperature regulation. These tests were made in the manner described, and the temperature regulatory functions of shivering, peripheral vasoconstriction and vasodilation as well as panting were normal. The threshold temperatures fell within the normal range, vasoconstriction and vasodilation rates were normal, and the type of shivering exhibited was that of a normal animal. The panting mechanism was also normal as judged by the respiratory rate-time graphs and the temperature-time graphs. The

numerical and graphical results of these tests are similar to those of normal dogs reported in the previous paper (Hemingway, 1940) and need not be reproduced here. In general, the tests showed normal temperature regulation.

Effects of locally heating the brain. Figure 3 shows the result of locally heating the anterior hypothalamus of a dog resting in a cool but not uncomfortable environment. Before heating, the ear vessels were constricted

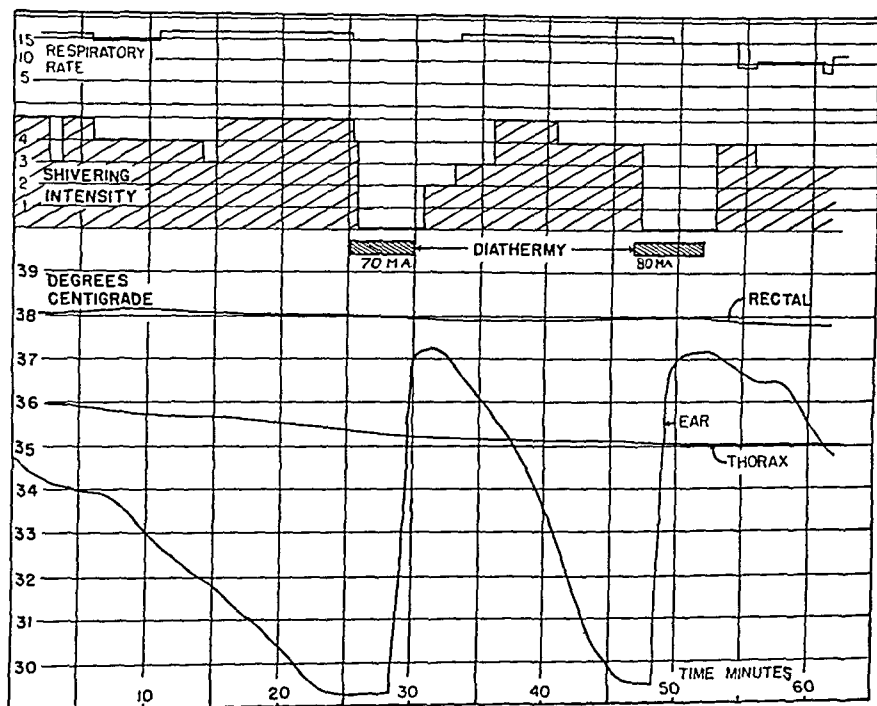


FIG. 3. Effect of locally heating the anterior hypothalamus: Anterior hypothalamic heating causes a sudden cessation of shivering and a rapid peripheral vasodilation. There is no evidence of panting.

as denoted by the low ear temperature of 29–31°C. The animal was shivering rather vigorously. When the diathermy heating current was turned on shivering ceased within one minute and the ear vessels dilated as indicated by the sudden rise of ear temperature. This effect occurred with a heating current of 70 mA and a high frequency voltage of 11.5 v, the heat production rate being 0.8 W. There was no change of trunk peripheral temperature nor rectal temperature. Increasing the current to a value as high as 200 mA did not produce any change in respiratory rate. When the posterior hypothalamus was heated in a similar experiment on another dog (Fig. 4) there was a slight reduction of shivering and no vasodilation with heating rates increased 3- to 4-fold. Drowsiness resulted with posterior hypothalamic

heating and shivering became reduced only when the animal fell asleep. In some cases sleep continued for 5 to 10 min. after the diathermy current had been discontinued and during this period there was reduced or no shivering. As soon as the animal awoke vigorous shivering continued. This was in sharp contrast to the shivering response when the anterior hypothalamus was heated, in which case shivering stopped and started within 1 min. of onset and cessation of the diathermy current.

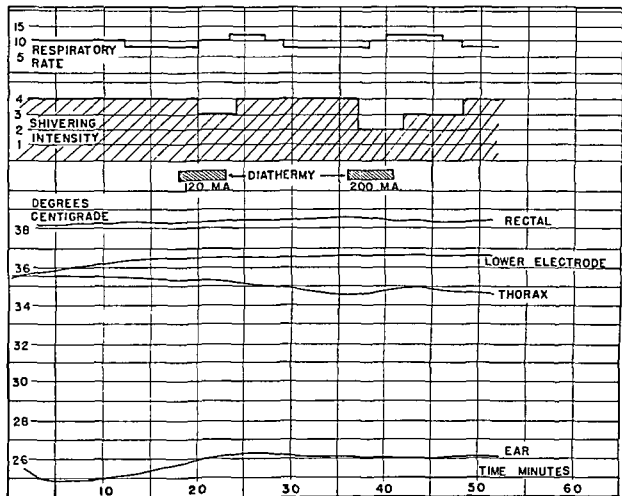


FIG. 4. Effect of locally heating the posterior hypothalamus. A relatively high current value causes a slight reduction in shivering but no peripheral vasodilation. There is no evidence of panting.

DISCUSSION

Quite different physiological effects are produced when the anterior and posterior hypothalamic regions are heated without changing peripheral and rectal temperatures. Shivering is almost instantaneously suppressed and peripheral vasodilation rapidly follows localized anterior hypothalamic heating. When the posterior hypothalamus is heated under similar conditions there is only a slight reduction of shivering and no peripheral vasodilation. This finding furnishes additional evidence to the theory that the "center" for heat loss is located in the anterior hypothalamus above the optic chiasm. It also proves that this region can stimulate the heat loss mechanisms in response to temperature changes of the cells of that region. In this respect

it behaves like the respiratory center of the medulla oblongata which is stimulated by changes in the carbonic acid content of the blood but differs in the fact that the hypothalamic stimulus is physical rather than chemical. The anterior hypothalamus may also serve as a coordinating and integrating center for nerve impulses from peripheral thermal receptors but further investigations are needed to separate more distinctly the anatomical and physiological mechanisms of reflex and central control before conclusions can be drawn.

Uprus, Gaylor and Carmichael (1935) made observations on the body temperatures of patients at which shivering started and stopped. They reached the conclusion that cessation of shivering was an inhibition evoked by a central stimulus. Our results obtained by an entirely different type of experiment are in accordance with their conclusions.

That the anterior hypothalamus is much more responsive to local heating than the posterior hypothalamus when heat loss mechanisms are involved is indicated by the following considerations. The anterior hypothalamic electrode of the dog whose experimental data are given in Fig. 3 was located mainly beneath the optic chiasm. When the tissue beneath the electrode was heated by diathermy the chiasma tissue would have the highest temperature while the hypothalamic tissue above and further from the electrode would be only slightly warmed. In spite of this intervening tissue a diathermy current of only 70 mA produced the striking changes of Fig. 3. When the posterior hypothalamus was heated a current of 120 to 200 mA, and hence producing 4 to 5 times as much heat, caused only a slight reduction in shivering, and this electrode was in immediate contact with hypothalamic tissue. One can conclude from these observations that the hypothalamic heat loss "center" is located just above the optic chiasm and is at some distance from the surface of the posterior hypothalamus.

	To arrest shivering	To cause peripheral vasodilation
Surface heating	2.26 Kcal.	9.0 Kcal.
Hypothalamic heating	0.013 Kcal.	0.032 Kcal.
Ratio	174	281

An advantage of diathermy heating is that the heating rate can be accurately controlled and measured. In the tests for normality of temperature regulatory function the dogs were heated by large diathermy electrodes applied to the skin with a heat dosage equal to the basal metabolic rate. In order to show the sensitivity of central heating in comparison with surface heating the heat production in Calories required to induce shivering and vasodilation by (i) hypothalamic heating and (ii) surface heating with large electrodes has been computed for the dog of Fig. 3. If one considers the anterior hypothalamus as a physiological thermostat, then hypothalamic

heating is equivalent to applying heat directly to the thermostat control whereas heating the entire animal raises the thermostat temperature only when the entire system is raised in temperature. These values prove beyond all doubt the existence of a thermal sensory region in the anterior hypothalamus.

One rather astonishing fact was the absence of panting. The experiment had been originally planned to measure this expected result of hypothalamic heating and all the more so after the appearance of the work of Magoun, Harrison, Brobeck and Ranson (1938) who produced panting in anesthetized cats by diathermy heating of the anterior hypothalamus using bipolar Horsley-Clark needles as electrodes. Our results indicate that either panting requires a more powerful heat stimulus than was given (with currents exceeding 200 mA the dog shows signs of discomfort) or that the sensory center for panting is located further from the hypothalamic surface than the shivering inhibitor and thermal vasodilator centers. This latter suggestion is supported by the brain transection experiments of Lilienthal and Otenasek (1937), *i.e.*, that in cats a region high in the thalamus had to be intact and in connection with lower parts of the central nervous system for panting to occur.

At the beginning of the experimentation it had been intended to complete the work by anesthetizing the dogs and heat coagulating the brain tissue beneath the electrodes by a massive diathermy current. The heat coagulated area would then have given an exact anatomical outline of the heated tissue. This procedure was abandoned for the following reason. If the brain tissue had been heat coagulated a post mortem examination would not have revealed whether or not the brain had been normal when the mild heating experiments had been performed. Anatomical inspection of the brains revealed that the hypothalami of the animals used were normal in all respects.

SUMMARY

Small gold foil electrodes, 3×6 mm. in size approximately with insulated thermocouple wires attached, were placed on either the anterior hypothalamus or the posterior hypothalamus of dogs by a subtemporal approach to the base of the brain. The free ends of the electrode wires were brought to a subcutaneous position on the skull and the dog allowed not less than one month to recover from the operation. Tests were made to determine if the temperature regulatory functions of shivering, panting, and peripheral vasoconstriction and vasodilation were normal. When these functions were normal the brain was heated locally by diathermy current from the brain electrode in a controlled environment. Heating the anterior hypothalamus caused inhibition of shivering and vasodilation. Heating the posterior hypothalamus produced sleep and a slight decrease of shivering intensity. Panting was not induced by local hypothalamic heating. The results prove the existence of centers for shivering inhibition and thermal vasodilation in the

anterior hypothalamus which are motivated by local brain temperature changes without changes of general body temperature or peripheral temperatures. Postmortem examination of the hypothalamus revealed that no hypothalamic structures had been injured in any way by the experimental procedures.

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RÔLE OF NEOCORTEX IN REGULATING POSTURAL REACTIONS OF THE OPOSSUM (*DIDELPHYS VIRGINIANA*)*

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KNOWLEDGE of the rôle played by the cerebral cortex of various mammals in the control of somatic motor responses has been obtained in many instances by a study of the deficiencies resulting from partial or complete decortication (2, 9, 11, 13). The easily tested placing and hopping responses have been employed to advantage in such comparative studies (1, 2, 3, 4, 6, 18, 19). This work has furnished a good demonstration of progressive corticalization of control since the deficiencies observed in higher forms are much more extensive than are those resulting from comparable cortical lesions in species which are generally considered to represent a lower level of development.

The opossum (*Didelphys virginiana*) represents an order of mammals which possesses characteristics quite different from those of the mammals previously studied in this way. There are also some similarities. Movements of the facial and limb musculature of this marsupial can be produced by electrical excitation of certain areas of the cerebral cortex (7, 8, 12, 15, 16, 20). Opossums likewise possess hopping and placing responses and are capable of many reactions requiring a high degree of muscular coordination. It was felt that a study of the rôle played by the cortex in the control of these specific somatic motor responses would yield some interesting results.

METHODS

A litter of 10 young opossums was obtained just when they had begun to leave the pouch. They consequently must have been at least 90 to 100 days old. Their reactions were tested at this time, electrically excitable areas of the neocortex of one hemisphere were delimited and various portions of the cortex then ablated. Six weeks later, during which interval the animals' reactions were tested weekly, 6 opossums were again anesthetized and the normal hemisphere exposed and stimulated. The entire neocortex of that hemisphere was extirpated in two animals at this time; the other animals were autopsied. Two surviving opossums were kept for one additional month. Cortical remnants were again stimulated after this period and the brains and spinal cords preserved for further study.

Electrically excitable areas of the neocortex of 8 adult opossums were delimited. Hemidecortication or various cortical lesions were made immediately following stimulation. The placing and hopping reactions of these animals were tested periodically. One month later two of them were again operated upon. Six weeks after the first operation those remnants of neocortex which remained in these 8 adult opossums were stimulated and the animals then autopsied.

Electrical exploration of the cortex of one hemisphere was performed in 10 young opossums which must have been 90–100 days of age, 6 animals 150–160 days old and 8 adults. Light ether anesthesia was used in all cases and the stimulator employed delivered a 60

* A preliminary report of this work was read before the American Physiological Society 1940 (*Amer. J. Physiol.*, 1940, 129: 319–320).

cycle sinusoidal wave. Voltage and current strength were recorded simultaneously during stimulation. A unipolar electrode was used for exploration of the cortex, the indifferent electrode being placed on the abdomen.

In ablating various small areas of the cortex, the tissue was first isolated from surrounding cortex with a sharp knife and then removed by means of a narrow-tipped pipette and gentle suction. In 4 adult animals we attempted to study degenerative changes in the pyramid by means of Marchi techniques. These preparations were not very satisfactory, possibly because the tissues were not obtained until 3 to 6 weeks after operation. The animals were not sacrificed sooner since we were chiefly interested in ascertaining the permanent state of their deficiencies.

RESULTS

Electrical stimulation. The results obtained from stimulation of the neocortex were for the most part similar to those reported by Herrick and Tight (8), Gray and Turner (7), Weed and Langworthy (16) and Vogt and Vogt (15). Even with relatively weak stimuli (1.5 v and 0.2 mA), however, definite isolated movements of the contralateral hindleg were elicited in 6 of the 10 young animals and in 2 of the 8 adults stimulated. Combined fore- and hindleg flexion was obtained in 12 of the 18 preparations. These hindleg movements were elicited from similar areas on the hemispheres of the several animals. A foreleg area lying just rostral to the region from which hindleg movements were obtained was more extensive and by far the most easily defined excitable area of the cortex. Extension of various toes, movements of the wrist, flexion and extension of the arm were also elicitable. Tail movements could occasionally be evoked by stimulation of the dorsal surface or the medial aspect of the hemisphere near the hindleg area. In 5 animals a small area on the lateral portion of the hemisphere gave rise to movements of the contralateral ear. Approximately a third of the animals exhibited respiratory acceleration when the cortex just rostral to the orbital fissure was stimulated. In addition to these rather clearly localizable effects other responses occurred such as movements of the nose, vibrissae or lips, rotation of the head, masticatory movements and closure of the contralateral eye. In some instances stimulation of a small area caused movement of the nostrils toward the contralateral side and excitation of a neighboring area provoked a movement toward the side stimulated. Contractions of facial musculature most commonly occurred when the region rostral and lateral to the orbital fissure was stimulated. One of the major difficulties encountered in attempting to determine the precise boundaries of the area from which a specific movement could be obtained was the fact that a given area failed to respond to repetitive stimuli. In many instances the movement could be evoked two or three times but after that the area remained unresponsive for several minutes. Foreleg movements, however, could be elicited at will and usually from a given point identical responses occurred. The results obtained in the adult were much less satisfactory than those given by the young opossums. Stronger stimuli were required, fewer isolated movements occurred and some responses frequently seen in the young animals did not appear in the adult. Figure 1G and H presents a diagrammatic summary of the results obtained in one young and one adult opossum.

Normal reactions. A study of certain normal somatic-motor reactions of these animals and the effects of various cortical ablations demonstrated that the electrically excitable area exerts some control over these responses. The opossum has hopping and placing reactions which are similar to those of other mammals. They are, however, much slower, less easily evoked and less exact than those of higher forms. In certain species special developments apparently accentuate the importance of some of these responses but in other forms postural or locomotor peculiarities render certain placing and hopping reactions less essential. Because of their highly developed anti-gravity mechanism, their posture and method of locomotion, dogs and cats are more dependent on the hopping responses than is the opossum. Like rats and monkeys, opossums tend to rotate their feet and grasp objects with which they come into contact. The arboreal life of this animal requires such placing reactions but their ready elicitation interferes with the elicitation of the hopping responses and modifies certain other placing reactions. The tendency of opossums to become absolutely passive also adds to the difficulties of adequately studying their postural responses.

Opossums show fairly good visual placing and in blindfolded animals the feet tend to place when the vibrissae come in contact with an object. The head and snout, however, are so long and the forelegs so short that the feet cannot be placed before the chin touches if the animal is held by its hind parts and lowered toward an object. If held parallel to a table edge an animal will frequently reach sideward and grasp the table. This occurs in the blindfolded opossum when the vibrissae touch.

If the forelegs of an opossum are held until the chin is brought in contact with the edge of a table both feet are raised and placed beside the jaws or the table edge is grasped. In executing this response the feet are frequently lifted higher than necessary and the reaction is much slower than that of a cat or rat (1, 4) although it is much faster than the alligator's response (3). The legs practically never extend, as in the cat, to lift the fore parts into a standing posture.

When the fore- and hindfeet are brought in contact with an object which the animal cannot see they are lifted and placed on the object. The response is slow and the feet are lifted unnecessarily high. Frequently the feet are rotated and the object is grasped. The latter practically always occurs when the sides of the feet make contact first. If any leg is forced into an awkward position or pushed off the edge of a table on which the animal is standing the leg is slowly but immediately replaced in a normal position.

The fore- and hindlegs show hopping reactions in response to adduction or strong abduction. The animals frequently grasp and cling to any irregularities of the surface on which they are being tested. In addition they occasionally endeavor to keep their legs in a normal relationship with the body by a type of shuffle which can be roughly described as a sideward movement of the foot by pivoting on the heel and then on the toes. The hopping reactions which do occur are more ponderous and much slower than those of

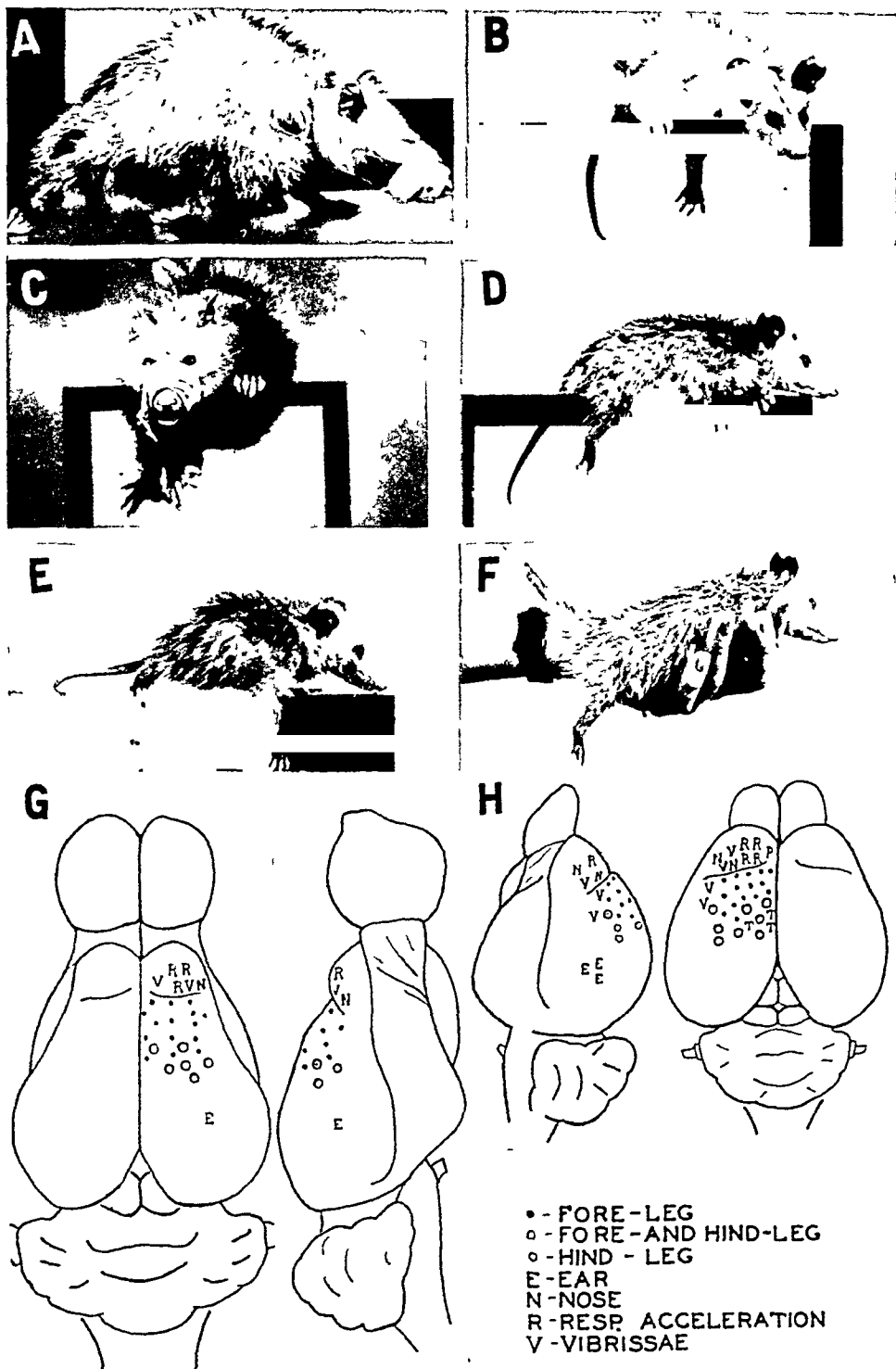


FIG. 1. (See next page for legend.)

higher forms. These reactions as well as the placing responses described above are fully developed in 90-100 day-old opossums.

Effects of cortical ablations. Unilateral ablation of the electrically excitable areas from which leg movements were obtained in 2 young and 4 adult animals resulted in a complete loss of contact placing responses in the limbs contralateral to the lesion. Proprioceptive responses were still present though probably somewhat impaired. Strong retroflexion of a limb caused it to be placed in an approximately normal position. Chin placing was abolished (Fig. 1C) and the legs frequently assumed or could be placed in abnormal attitudes. In walking the animals frequently stepped or stood on the backs of their affected feet (Fig. 1A) and when standing on a bar or near the edge of a box one or the other affected leg commonly was not replaced if it slipped or was pushed out of position (Fig. 1B, D, E, F). Visual placing was likewise impaired and the hopping reactions were decidedly subnormal. Bilateral removal of this area resulted in bilateral deficiencies. Young animals were much more active than were the adults and consequently seemed to be less affected by the lesions. Careful tests revealed no detectable differences in the deficiencies. Young animals did not recover their placing responses and the hopping reactions were permanently defective in both young and old opossums (5).

Complete unilateral ablation of the neocortex in 4 young and 2 adult opossums produced no greater deficiencies in these postural responses of the contralateral limbs than had been observed to result in other animals from unilateral removal of the sensorimotor area alone. Visual impairment was, of course, greater in those animals from which the occipital portions of the neocortex had been extirpated. In one young and one adult animal the entire neocortex of one hemisphere and the electrically excitable area of the other side were ablated. The placing and hopping reactions of the limbs of the two sides were equally deficient.

In one young opossum an endeavor was made to remove the foreleg area alone and in two the area from which hindleg movements had been evoked. All three showed both fore- and hindleg deficiencies. Although they could be distinguished from animals with more extensive lesions we were not al-

EXPLANATION OF FIG 1

A Postural abnormalities in an adult opossum following ablation of the electrically excitable areas of neocortex of the left hemisphere. Animal is standing on dorsum of right forefoot. The right hindleg is abnormally abducted

B and D Young opossum which shows defective placing responses in the right legs following a left hemidecortication

C. Failure of the right legs of the animal shown in A to place when chin is rested on a bar

E and F Failure of the foreleg and hindleg to place normally following ablation of the left sensorimotor cortical area. The postures were assumed by the animal

G. Diagram of the brain of an adult opossum showing the points from which various movements were obtained on electrical stimulation.

H Similar diagram of the brain of a young animal

ways able to tell by testing the responses which animal possessed the "fore-leg" lesion.

Extensive lesions in the occipital cortex of 3 young and one adult opossum produced visual deficiencies but no impairment of the contact placing or the hopping responses. In one young opossum the neocortex of one hemisphere and the occipital cortex of the other were extirpated. Reactions in the legs opposite the intact electrically excitable area were normal. The cortex rostral to the orbital sulcus of one hemisphere was ablated in another animal. No observable deficiencies resulted.

An abnormality of the grasping responses was noticed in the 3 animals with unilateral ablations of the electrically excitable area. Objects with which the feet came in contact were not grasped as frequently with the affected as with the normal feet. The deficient feet did on occasion grasp objects quite firmly. A finger or a small rod placed in an animal's normal hand was grasped but if one endeavored to lift the animal in this way it let go. If the affected hand gripped the finger or rod the animal could be lifted from the ground and it usually hung on until some extraneous activity caused it to lose its hold. It thus appeared that the affected hand grasps less readily but having done so it cannot let go with normal ease. The hindleg showed this same tendency, but less definitely. This abnormality of grasp response should be studied more thoroughly before being compared in any way with the grasp reflex of monkeys (13).

In the young animals which were kept for several weeks after partial or complete ablation of the sensorimotor cortex of one hemisphere changes were observed to have occurred in the pyramidal tract above the decussation. The ipsilateral pyramids of the 3 animals in which small lesions had been made were reduced in size. Complete hemidecortication or removal of the electrically excitable area caused an almost complete disappearance of the ipsilateral pyramidal tract. Our Marchi preparations like those of Turner (14) did not show degenerating pyramidal fibers below the upper cervical region of the cord but the preparations were rather poor. We have no basis for judging whether a pyramidal or extrapyramidal system or both are responsible for the effects obtained by electrical stimulation of the cortex. We do know that the deficiency-producing ablations do cause degeneration of the grossly visible pyramidal system. This does not indicate, however that the defects observed were due to this alone.

DISCUSSION

The placing and hopping reactions of opossums are slower and less exact than those of higher forms. They are, however, controlled in part by the cortex. As in the monkey, dog, cat, rabbit and rat contact placing is absent following ablation of electrically excitable cortical areas. The opossum's contact placing reaction, consequently, is just as dependent on the cortex as is that of the monkey. On the other hand, cortical removals do produce less change in the hopping reactions than in higher mammals. It, therefore,

seems justifiable to state that the cortical control of these postural reactions is less highly developed in this marsupial.

In the opossum as in the rabbit a hindleg cortical representation is not easily demonstrated but in 11 of the 17 animals stimulated, discrete hindleg movements were obtained. In 8 cases the hindleg flexions were not associated with foreleg movements. The existence of a cortical control of hindleg reactions was likewise demonstrated by the fact that ablation of the electrically excitable cortical areas abolished the hindleg contact placing responses and rendered the hopping reactions definitely subnormal.

Electrical excitation of the cortex produced more responses in the young animals than in the adults studied. There may be certain periods of an animal's development during which the system stimulated is more susceptible to electrical excitation. Possibly due to the smallness of the brain in young animals or the greater permeability of the tissue a larger number of sensitive cells lie within the effective field of the electrode at any given strength of stimulation.

SUMMARY

Electrical stimulation of the neocortex in 17 opossums revealed various areas from which movements of the contralateral facial, foreleg and hindleg musculature could be obtained.

The placing and hopping responses of the opossum are much slower and less exact than those of phylogenetically higher forms.

These postural responses are controlled in part by the electrically excitable areas of the neocortex. Ablation of this sensori-motor area of one hemisphere produces deficiencies in the responses of the contralateral foreleg and hindleg.

The deficiencies in postural adjustment resulting from cortical lesions are less extensive than those resulting from ablation of similar areas of the cortex in higher forms.

Visual responses are affected by removal of the visual cortex but with this exception lesions in non-excitable portions of the cortex cause no deficiency of the postural responses studied.

Extirpation of a portion of the sensori-motor area produces deficiencies which are less extensive than those resulting from ablation of the entire area. Bilateral ablation of electrically excitable cortical areas results in bilateral deficiencies.

Unilateral removal of the electrically excitable area alone produces just as great deficiencies in the placing and hopping responses of the contralateral legs as does ablation of the entire neocortex of one hemisphere.

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CHEMICAL CONSTITUTION AND ANESTHETIC POTENCY IN RELATION TO CORTICAL POTENTIALS

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IN A PREVIOUS PAPER (Beecher and McDonough, 1939) the cortical electric response to 17 anesthetic agents was reported at two levels of anesthesia, as was the response following sciatic stimulation at each level. While these 17 agents represent many classes of compounds, in a few cases, several members of the same chemical family are present. From observations made on these agents, it appeared desirable to study systematically the relationship of gradually changing chemical composition to cortical effect. The material at hand which was suitable for such comparison was of very limited extent and what there was represented rather extreme changes even within a family of similar compounds. Accordingly, for further study a simple class of anesthetic compounds was chosen, one in which the complexity could be gradually and systematically increased. A homologous series of aliphatic alcohols best filled the requirements.

More than 70 years ago the generalization concerning the relationship of the molecular weight of alcohols to their anesthetic potency which has since come to be known as the "Law of Richardson" was stated. Richardson said " . . . as the weight of the alcohol increases, as the carbon and hydrogen, but especially the carbon, increases, the narcotic action of the agent is increased." This has been supported by numerous subsequent studies. Evidence has also been obtained that the higher forms, the secondary and tertiary forms of the alcohols are more potent than the primary.

It is evident that an opportunity is thus offered for a double check of the possibility of a significant relationship existing between anesthetic potency and cortical response, as well as an opportunity for studying the effect of gradually changing chemical structure of the anesthetic agents on the cortical response. The purpose of the study was to subject these possibilities to experimental test.

METHODS

Animals Cats were used in all experiments. The right posterior sigmoid gyrus of the cortex was exposed. Activity was recorded from the region of the sensory area in all cases.

Anesthetic agents Eleven alcohols were employed as anesthetic agents. In the series of normal alcohols the seven agents, methyl, ethyl, propyl, butyl, amyl, hexyl and heptyl were used, and in addition to this series, secondary propyl, tertiary butyl, tertiary amyl, and tertiary hexyl were administered.

Levels of anesthesia Two levels were used. These were determined by the flexion reflex evoked on electrical stimulation of the central end of the cut left sciatic nerve. The stimulating electrodes consisted of silver wires encased in a rubber tube, the cut sciatic nerve was inserted into this. These electrodes were connected with the secondary coil of a Har-

vard inductorium. In circuit with the primary (activated by a 1.5 v. dry cell) was a hand operated mercury contact key and a string galvanometer signal device. The stimuli were make and break shocks spaced from one to three seconds apart, and usually followed by a rapid series of six or eight shocks (by hand). The ipsilateral nerves to the hamstring muscles were left intact so that a flexor response of the lower leg might occur and be recorded on a smoked drum. The strength of the stimulating current was adjusted to give approximately a maximal response. The two levels of anesthesia were arbitrarily chosen and studied in detail: (i) The lightest anesthesia it was possible to work with without producing a disturbing generalized muscular response, on stimulating the sciatic nerve, and (ii) the level at which the flexion reflex just disappeared. These two levels cover a wide anesthesia range. These two levels will be referred to as light and deep anesthesia. Records of activity at the light level are characterized by greater voltage than those obtained at the deep level and are easier to examine. On the other hand, the light level is somewhat less precise than the deep. Presumably this accounts for the greater variability of the data at the light level than at the deep, as shown in the Table.

Electrodes. The concentric cortical electrodes described by Beecher, McDonough, and Forbes (1938) were used. These are made of silver supported on a hard rubber base. The grid lead is spike-shaped and protrudes 2 mm. beyond the base. It is everywhere insulated except at its tip. At the base it is surrounded by the ground lead, a circular band 1 mm. in width and 6 mm. in outside diameter. The electrodes were freshly chlorided electrolytically each time before use. With these, potential differences were recorded between the surface of the cortex and 2 mm. deep in the interior. When these electrodes are used the ground lead fills a large part of the opening in the skull made necessary in order to identify the desired cortical position. Herniation of the brain is thus in large measure prevented and the arterial and respiratory pulsations minimized.

Amplifier. The potential differences between the grid and ground leads were amplified by the direct coupled apparatus described by Forbes and Grass (1937) and were recorded on film with a Hindle string galvanometer. Ten milliseconds units were recorded by a timer on one margin of the film. The signal device recorded the stimuli on the opposite margin.

RESULTS AND DISCUSSION

The standard deviation of the mean* frequency has been calculated in all cases. Frequencies per second of the cortical waves are given. When checked they rarely differed by more than two or three waves per sec. Every deflection was counted as a wave. Care was taken to avoid 60-cycle interference. In each observation activity was usually recorded continuously for 5 to 10 sec. A full second was counted in each case; the seconds, though arbitrarily chosen for counting, were spread out as well as possible over the entire experiment at the given level of anesthesia. The data on frequencies are presented in the Table.

All of the alcohols behaved in regard to their associated electrical cortical activity, as expected, like the volatile anesthetic agents (cf. Beecher and McDonough, 1939). In other words, the pattern was characterized by rapid, fine waves. Sciatic stimulation under light anesthesia produced an increase in voltage of the waves. It also produced a smooth cumulative flexion reflex suggesting that long circuiting of impulses in the nervous system is little curtailed (cf. Beecher, McDonough and Forbes, 1939). No "secondary discharge" (Forbes and Morison, 1939) was definitely elicited in response to

* This is derived by the standard statistical formulae:

$$\text{S.D. (individual)} = \sqrt{\frac{\sum D^2}{N-1}} = x; \text{S.D. (mean)} = \frac{x}{\sqrt{N}}$$

To test for significant difference: $M_1 - M_2 > 2\sqrt{\text{SD}_1^2 + \text{SD}_2^2}$

sciatic stimulation. With the heavier alcohols, notably heptyl, suggestive discharges were obtained. These were not definite. Previously, Beecher and McDonough (1939) had reported secondary discharges with amylene hydrate (tertiary amyl alcohol). These were found when the components* of "avertin" were being studied. In the present experiments better grades of alcohols were obtained from the Eastman Kodak Company. In this case, unmistakable secondary discharges were not obtained with any of the alcohols.

Table

Anesthetic Agent Alcohol	Expts		Counts	Counts	Mean Frequency per sec With Standard Deviation	
	L	D	Light	Deep	Light Anesth.	Deep Anesth.
Methyl	5	5	80	60	31.5 \pm 0.3	30.0 \pm 0.3
Ethyl	6	6	82	77	29.9 \pm 0.3	30.1 \pm 0.8
n-Propyl	3	3	58	52	31.1 \pm 0.4	29.7 \pm 0.4
n-Butyl	3	3	60	46	29.9 \pm 0.3	28.5 \pm 0.5
n-Amyl	3	3	42	44	26.8 \pm 0.6	25.5 \pm 0.5
n-Hexyl	3	3	49	43	23.6 \pm 0.4	21.0 \pm 0.4
n-Heptyl	3	3	51	54	25.4 \pm 0.4	21.4 \pm 0.5
2-Propyl	3	3	45	32	25.8 \pm 0.5	26.4 \pm 0.6
3-Butyl	3	3	45	30	27.1 \pm 0.4	23.1 \pm 0.4
3-Amyl	6	4	65	58	22.0 \pm 0.6	21.9 \pm 0.5
3-Hexyl	3	3	52	41	20.6 \pm 0.4	19.1 \pm 0.4

When 4 carbon atoms are exceeded, the alcohol becomes so toxic it is surprising that the relationships elicited held as well as they did. Presumably with the enormous increase in toxicity, side reactions occur which may overshadow the anesthetic action. Studies of this nature usually are terminated with examination of the 5 carbon alcohol, amyl. It has been interesting, however, to continue the study through the 7 carbon alcohol.

While the "Law of Richardson" does not need further support as far as the simple alcohols are concerned, the data contained in Fig. 1 (obtained during the preparation of the animals for the present experiments) have been included for comparison with Fig. 2 where the relationship of specific alcohol to total frequency per second of cortical waves is shown. The rate of change of the curve in Fig. 2 bears an inverse relationship to that of Fig. 1. The total frequency of cortical waves slows with increase in the number of carbon atoms (increase in molecular weight), that is, with increase in anesthetic potency. This suggests a relationship between the total frequency of cortical waves and anesthetic potency, but so far this might only indicate a parallelism between total wave frequency and molecular weight, since molecular weight and potency are related for these compounds. It is possible to go farther than this and to bring out strong evidence that the total cortical wave frequency is related to anesthetic potency rather than molecular weight of the anesthetic agent.

* Tribromethanol in amylene hydrate

Certain alcohols can be considered as derivatives of methyl alcohol $\text{CH}_3 \cdot \text{OH}$, called carbinol for this purpose. Normal butyl alcohol $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$, for example, contains the group $-\text{CH}_2 \cdot \text{OH}$; it may be considered as a mono-substitution product of carbinol, and is called a primary

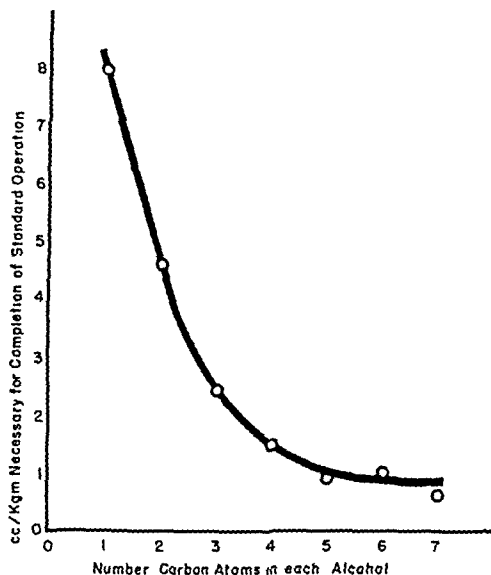


FIG. 1. This demonstrates the increased potency of the alcohols with increase in the number of carbon atoms contained. ("Law of Richardson").

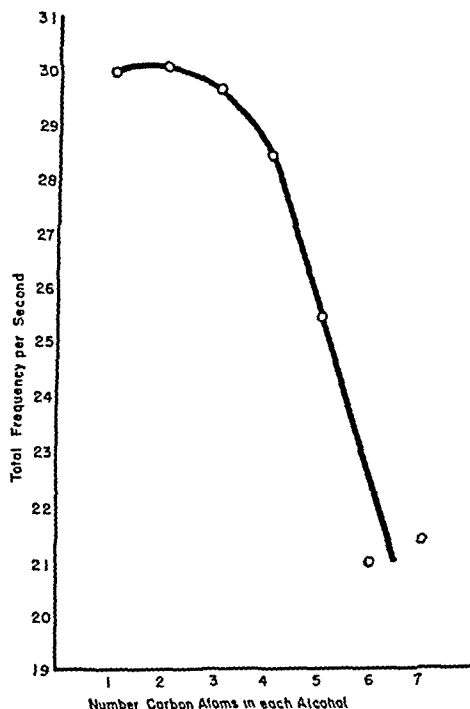


FIG. 2. The relationship of each alcohol anesthesia to total frequency per second of cortical waves. Level of deep anesthesia.

alcohol. Similarly, secondary butyl alcohol $\begin{matrix} \text{CH}_3 \\ \text{C}_2\text{H}_5 \end{matrix} \rangle \text{CH} \cdot \text{OH}$ contains the group $>\text{CH} \cdot \text{OH}$; it may be considered as a di-substitution product of carbinol. Likewise tertiary butyl alcohol $\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \\ \text{CH}_3 \end{matrix} \rangle \text{C} \cdot \text{OH}$ contains the group R_3

$\text{C} \cdot \text{OH}$ and represents a tri-substitution product of carbinol. In these three compounds the variation from one to the others is in structural formula. The molecular weight, the number and kind of atoms remain the same in the primary, the secondary and the tertiary alcohols.

As already pointed out, the secondary and tertiary forms of alcohols are known to have a higher potency than the primary form. This provides another opportunity to observe if the total frequency slows with increased anesthetic potency. Again, a parallelism is shown. (See the Table and Fig. 3.) With the more potent form of the alcohol, the frequency is significantly slower, although in this case the molecular weight and atomic composition

were unchanged. Only the structural form was changed. A significant difference exists in the cases presented in Fig. 3. This has been demonstrated from the standard deviation of the means *

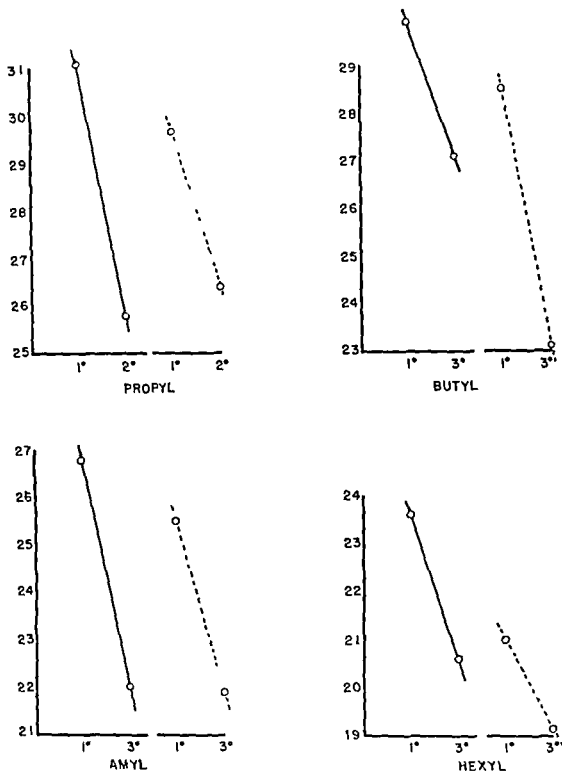


FIG 3 The form of the alcohol is shown on the abscissa, the total frequency per second of cortical waves on the ordinate. The continuous line represents light anesthesia, the broken line deep anesthesia

* n propyl and 2°-propyl $5.3 > 1.3$

n -butyl and 3°-butyl $2.8 > 1.0$

n amyl and 3°-amyl $4.8 > 1.7$

n hexyl and 3° hexyl $3.0 > 1.1$

As shown here, the difference of the means for the two propyl alcohols, for example, is 5.3, much more than twice the square root of the sum of the standard deviations of the means squared, in this case, 1.3. This is true also for the three other alcohols shown, and the changes are significant

It would be desirable to have an analysis of the step by step changes produced in the pattern of waves, an analysis which possibly might demonstrate the effects of each additional CH_2 group. It is hoped that such a study can soon be undertaken. The present study is limited, however, to an objective demonstration of the effects of increased potency on one characteristic of central nervous system activity: total frequency of cortical waves. Heretofore, potency has been considered in regard to variations in depth produced by a given quantity or type of agent or it has been considered in regard to variations in quantity or type of agent to produce a given depth of anesthesia. Here, potency has been studied from the point of view of central nervous system activity at a *constant level* of anesthesia produced by a number of different agents. This is a new approach to an old problem which promises to lead eventually to a better understanding of the nature of anesthetic potency and of anesthetic action.

SUMMARY

The cortical electric response to anesthesia from eleven alcohols has been studied in cats in regard to the effects of an increase in carbon atoms in the alcohols and an increase in potency, to the total frequency per second of cortical waves: the frequency becomes progressively slower with increasing molecular weight (increased number of carbon atoms) of the alcohols (Table, and Fig. 2). The rate of change of the frequency curve (Fig. 2) is the reciprocal of that of the potency curve (Fig. 1). That this slowing is due to an increase in anesthetic potency rather than to an increase in molecular weight *per se* is shown by the fact that the total frequency is also slower when the secondary or tertiary forms of the alcohols are compared with the primary (Fig. 3), thus frequency appears to bear a definite relationship to the anesthetic potency of the alcohol, even when molecular weight and atomic composition (but not structure) are maintained unchanged. A new approach to study of the meaning of anesthetic potency is presented.

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OCULAR MOVEMENTS FROM THE OCCIPITAL LOBE IN THE MONKEY*

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HISTORICAL INTRODUCTION

ALTHOUGH Ferrier (6) and Luciani and Tamburini (12) reported ocular movements upon electrical excitation of the cortex of the angular gyrus and occipital lobe, Schafer (15) was the first to study these movements in detail. His observations based on the stimulation of the lateral, medial and inferior surfaces of the occipital lobe of the monkey led him to suggest certain connections between the cerebral visual area and the retina—connections which subsequent neuro-anatomical studies have largely confirmed. Later reports have added little to his description. However, all of the investigations were made when little was known of the anatomical limits of the striate cortex or of its connections. The present study aims to correlate physiological and anatomical knowledge of the occipital cortex.

MATERIAL AND METHODS

Twelve adult monkeys (*Macaca mulatta*), convalescent from operations on the spinal cord, were used for this investigation. In none had any operative procedure been carried out on the cerebrum.

Ether or divinyl ether (vinethene) was used as the anesthetic for the operative procedures. A midline incision of the scalp allowed sufficient exposure to remove the calvarium over the occipital, parietal and in some cases the frontal lobes, on one or both sides. The dura was then widely opened and the cortex covered with cotton moistened in warm normal saline.

The cortex was stimulated with bipolar electrodes separated 2 mm using a sixty cycle alternating sine wave current so arranged that any desired strength of stimulus might be applied.

The ocular movements were observed by one of us seated in front of the animal. Records of the movements were made on bromide paper by passing a suture through the pericorneal conjunctiva after cocaineization and attaching it to an upright flexible thin arm which interrupted a beam of light focused upon the camera. Graphic records of the eye movements were not very satisfactory, however, due to the difficulty in registering two-dimensional movements.

RESULTS

Stimulation of the cortex of the occipital lobe and of the adjacent angular gyrus produced slow conjugate deviation of the eyes to the opposite side with or without up or down movements. The response usually had a latency of 1–2 sec. and was 1–2 sec. in completion. The movements were usually smooth, but in the lightly ether-anesthetized animals they were frequently nystagmoid in character. The deviation of the eyes persisted as long as the stimulation was maintained—for periods up to half a minute.

The strength of stimulus necessary to produce these movements varied

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somewhat in different animals but was consistently higher than that required to cause deviation of the eyes by excitation of area 8. Not all points had the same threshold—those about the external calcarine fissure usually



FIG. 1. Photograph of the lateral (A) and medial (B) surfaces of the parieto-occipital lobes of the monkey brain (*Macaca mulatta*). The numbers represent points of application of the stimuli from a composite study of the entire series. The strengths of stimulation varied from 1-5 v. R.M.S. depending upon the site of stimulation, the condition of the animal and the depth of anesthesia. AS, sulcus parieto-occipitalis externus; CC, corpus callosum; CM, sulcus callosomarginalis; FC, fissura calcarina; IP, sulcus intraparietalis; IT, sulcus temporalis inferior; LO, sulcus occipitalis lateralis; PI, sulcus parieto-occipitalis internus; S, fissura sylvii; ST, sulcus temporalis superior; EC, fissura calcarina externa.

- 1, 2, 3, 5, 6, 7. Conjugate deviation of the eyes to the opposite side and downward.
4. Conjugate deviation of the eyes to the opposite side.
- 8, 10, 11. Conjugate deviation of the eyes to the opposite side and upward.
12. Conjugate deviation of the eyes to the opposite side and slightly upward.
13. Conjugate deviation of the eyes to the opposite side with an inconstant downward component.
14. Conjugate deviation of the eyes to the opposite side with an inconstant downward component. Slight pupillary constriction.
15. Conjugate deviation of the eyes to the opposite side with an inconstant downward component. Closure of the eyelids.
16. Widening of the palpebral fissures with slight upward movement of the eyes.
- 17, 18, 19. Slight conjugate deviation of the eyes to the opposite side.
- 20, 22. Nil.
21. After a latency of 2-3 seconds slight conjugate deviation to the opposite side.
- 23, 24. Conjugate deviation of the eyes upward and to the opposite side.
25. Conjugate deviation of the eyes downward and to the opposite side.
- 26, 29, 30, 32, 33, 34. Conjugate deviation of the eyes to the opposite side.
- 27, 28. Conjugate deviation of the eyes downward.
31. Conjugate deviation of the eyes to the opposite side and upward.

giving rise to movements with a lower threshold than points near the margin of the striate cortex. The parastriate cortex in general had a still higher threshold. Strengths of stimulation as low as 1.0 v. R. M. S. applied to the

striate cortex along the external calcarine fissure produced ocular responses (the threshold for area 8 was under 1 v R. M. S.).

Associated with the lateral conjugate movement of the eyes was frequently a downward or upward component and at times the movement was practically entirely vertical. Although stimulation of the peristriate area occasionally gave rise to upward or downward gaze, stimulation of the medial part of the convexity of the occipital lobe (striate cortex) consistently did so. Excitation of the striate cortex above the external calcarine fissure produced a conjugate deviation of the eyes downward with or without a lateral component. Stimulation below the external calcarine fissure caused conjugate upward movements of the eyes. It was possible to overcome an assumed upward gaze by stimulation just above the external calcarine fissure. By moving the electrodes along the striate cortex, from below to above the level of the external calcarine sulcus (from point 11 to point 5 on Fig. 1) one could cause the animal's eyes to sweep in a semi-circle from an upward to a downward gaze.

On the medial surface of the hemisphere stimulation above the calcarine fissure near the occipital pole produced downward gaze, and excitation below the calcarine fissure upward deviation of the eyes. Stimulation along the anterior part of the calcarine fissure produced conjugate deviation of the eyes to the opposite side with little up or down movement. Lateral movements of the eyes with occasional upward or downward components could be elicited by stimulation of the peristriate area on the medial surface of the hemisphere. After removal of the occipital operculum lateral deviation of the eyes with slight downward components were elicited by stimulation of the cortex of the annectant gyri with a stimulus slightly stronger than that necessary to produce the responses from the operculum.

It must be noted that we have observed on occasions upward deviation of the eyes from stimulation above the external calcarine sulcus. The significance of the observation is not apparent at this time. We have also seen conjugate deviation of the eyes to the ipsilateral side but so inconsistently that we cannot be certain the observation was not a chance movement of the eyes.

Other oculomotor phenomena were not of prime interest in this study. However, pupillary dilatation and constriction, and movements of the eyelids have occasionally been noticed.

DISCUSSION

Ocular movements resulting from stimulation of the occipital cortex have been noted by many investigators in a wide range of experimental animals including dogs (13), cats (12, 13), monkeys (1, 2, 3, 5, 7, 11, 14, 16, 17, 18) and chimpanzee (9). The majority of these papers describe only briefly the responses obtained from the most accessible part of the occipital cortex, namely the operculum. Only Schäfer (15) has explored the occipital lobe in detail and unfortunately he does not give protocols or illustrations

of his experiments. He states that when the electrodes are placed on the upper part of the occipital cortex, either on the lateral or medial surfaces the lateral deviation of the eyes is accompanied by a downward inclination. When they are placed on the posterior extremity of the lobe, its tentorial surface or the inferior part of the medial surface the deviation is accompanied by an upward inclination. From these observations Schäfer (15) suggests that: (i) The whole of the visual area of one hemisphere is connected with the corresponding lateral half of both retinae. (ii) The upper zone of the visual area of one hemisphere is connected with the upper part of the corresponding lateral half of both retinae. (iii) The lower part of the visual area is connected with the lower part of the corresponding lateral half of both retinae. (iv) The intermediate zone of the visual area is connected with the middle part of the corresponding lateral half of both retinae. What an amazingly accurate hypothesis formulated at a time when there was no agreement on the localization of the cortical visual center by all investigators!

That the external calcarine sulcus represents the horizontal meridian is not yet established anatomically today. Poliak (14) is indefinite on this point. Van Heuven (10) and Brouwer (4) place the horizontal meridian well above the external calcarine fissure. From the physiological standpoint the present experimentation favors the suggestion that the external calcarine sulcus is the cortical representation of the horizontal meridian. Unfortunately, few observations have been made on human beings. Foerster (8) failed to obtain eye movements upon stimulation of areas 17, 18, or 19 but elicited a visual hallucination of light in the lower half of the visual field upon stimulation of the superior lip of the calcarine fissure and a similar hallucination in the upper field by stimulation of the inferior lip.

The anatomical pathways for these eye movements have not been investigated in this study. Schäfer (15), however, has shown that the ocular movements produced by excitation of the occipital and temporal lobes are quite independent of the frontal eye fields, for their ablation does not alter the response. Nor does section of the corpus callosum change the reaction. Bernheimer (3) found that the responses were still elicitable after destruction of the superior colliculi and concluded that the pathways probably crossed under the aqueduct of Sylvius. The precise course of the fibers mediating the responses are, however, unknown.

Dusser de Barenne (5, p. 286) believes that the ocular movements obtained by a stimulation of areas 7a and 19a of the Vogts are not cortical in origin since they may be obtained after thermocoagulation of the cortex of these areas. He considers these responses due to excitation of subcortical fibers passing beneath these areas. We have considered this hypothesis as a possible explanation for the movements obtained from the striate cortex. In the acute preparation after thermocoagulation or ablation of the striate cortex, the responses are obtainable from the damaged cortex or white matter with only a slightly higher strength of stimulation than necessary

before the procedure. If, however, the animal is allowed to convalesce for a week or ten days to permit the projection fibers to degenerate and the cortex then restimulated, the specific responses are absent even with strengths of current two or three times the threshold of the adjacent normal striate cortex. It therefore, seems likely that the ocular responses elicited from the striate area are the result of cortical stimulation and not due to excitation of underlying fibers

CONCLUSIONS

Contralateral conjugate deviation of the eyes with lateral, or with either upward or downward components was obtained by stimulation of the occipital cortex. When the exciting electrodes were applied to the cortex of area 17 superior to the calcarine fissure, the movements tended to be lateral and downward. When applied below the same landmarks, the deviation was lateral and upward. The relationship of this finding to the projection of the retina on the cerebral cortex is discussed.

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NATURE OF THE FIRST VISIBLE CONTRACTIONS OF THE FORELIMB MUSCULATURE IN RAT FETUSES

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LITTLE is known about the physiological properties of the developing striated musculature of mammals at or near the time of its earliest contractility. Most of the existing observations have been incidental to studies of the ontogeny of behavior, or rather of reflex activity (1, 2, 15, 30). In such instances the characteristics of the visible muscular activity have been regarded, at least by implication, as directly reflecting the intrinsic physiological properties of the muscles involved. No consideration seems to have been given to peripheral structural factors that conceivably might modify the form of the muscular response.

This paper deals with the nature of the first visible forelimb responses to electrical stimulation in rat fetuses. Brief morphological correlations have been made. We have not observed somatic movements in any fetuses up to the 16th day of gestation, even when very strong currents were employed. The earliest visible contractions were secured from the forelimb musculature during the 16th day of gestation. Movements produced by contractions of the neck muscles have been elicited at about the same time, while the trunk and proximal hind-limb muscles become visibly reactive not long thereafter.

Most of the animals studied were white rats, but pied animals were used in a few experiments. According to the data collected by Donaldson (7), no appreciable differences between them would be expected. Twenty-five 15-day-old fetuses, all without perceptible response, were studied. These came from 5 litters (with estimated copulation ages of from 346 to 349 hours) and measured 9.5–12 mm. crown-rump length.

The data presented herein have been obtained from 12 fetuses of 7 different 16-day-old litters (with estimated copulation ages of from 368 to 378 hours), ranging between 12.5 and 14.5 mm. C. R. (nos. 10-2, 11-1, 19-1, 19-2, 20-1, 20-2, 20-3, 25-2, 101-1, 101-2, 101-3, 102-1). Some of these were asymmetrical in responsiveness. Furthermore, not all specimens of this size or age responded visibly to stimulation. Some of the 16-day litters contained both reactive and non-reactive fetuses measuring more than 12.5 mm. We have not observed contractions in fetuses smaller than 12.5 mm. C. R. One other litter of 3 animals (no. 5), estimated to be 371 hours old and measuring 12–12.5 mm. C. R., gave no perceptible response.

Visible, electrically induced contractions of the forelimb musculature of the rat therefore first appear some time during the latter half of the 15th

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or the first half of the 16th day of prenatal life. In view of the variability of responsiveness among our fetuses of the latter date, it seems likely that we have been studying specimens at or shortly after the point of transition. Windle *et al.* (30) obtained their earliest responses to electrical stimulation in rat fetuses 368 hours old, 11 mm. C. R. This age agrees with that of our own experience, but their fetuses were about 2 mm. smaller. Possibly this discrepancy is related to a difference in stocks of rats, but more likely is referable to dissimilarity in method of measuring the fetuses.

TECHNIQUE

The experiments were so conducted that the fetuses remained attached to the mother, with circulation intact. Their condition uniformly was good, body-color being used as the chief criterion. This appears to be a good index, because the normal pinkish color of the fetus rapidly changes to a purple whenever the circulation is obstructed or other anoxic conditions obtain.

The pregnant female rats (except those noted below) were anesthetized by subcutaneous injection of Nembutal (Abbott's Veterinary, no 8612, each cc containing 1 grain) in doses of 0.1 cc per 100 g of body-weight. The general condition of the mother rat was excellent throughout the experiment, with two exceptions (nos 16 and 25). Control experiments were carried out in order to ascertain whether the use of Nembutal suppressed or modified the muscular responses. Thus one of the 15-day pregnant animals was studied while under ether, and another after complete section of the thoracic spinal cord, without general anesthesia (over 3 hours being allowed for recovery from ether administered during spinal section). Two of the 16 day pregnant mothers (nos 101 and 102) were investigated after similar thoracic section, without subsequent anesthesia, by the senior author after his return to the United States. In all of these instances, however, the results obtained were identical with those of the other experiments on fetuses of the same ages. Hence the data secured from the 16-day controls have been included in this communication.

The anesthetized mother rat was tied to a board and her hindlegs and trunk submerged in a bath of physiological salt solution maintained thermostatically at or near a temperature of 37°C. The abdomen then was opened by midline incision and the uterus exposed. The fetus to be studied was removed, with amnion intact, from the uterus to an adjacent submerged shelf composed of several layers of gauze stretched on a wire frame. All experiments were carried out under fluid, the fetus usually lying close to the surface of the bath. Some fetuses were lifted out of the fluid and studied for special points. Most of the observations were made through a binocular dissecting (Greenough) microscope at enlargements up to 10 diameters. Afterwards, the umbilical cord was ligated and severed, the fetus measured under fluid with sliding calipers and finally preserved for histological study.

The fetuses first were observed while in the amnion. The latter then was removed, care being taken to avoid injury to the placental circulation. The fetuses were stimulated electrically through their skin. This was so thin that it offered little obstruction to a current, because when the skin was punctured and the stimulus applied to the muscles directly there was no appreciable difference in the response. Steel bipolar electrodes with fine points slightly less than 0.5 mm apart were used. These were insulated to the tips to prevent undue loss or spread of current. Faradic current from an inductorium was applied to most of the 15-day litters and to 16 day litters nos 5, 10, 11, 101 and 102 (these including responsive fetuses 10-2, 11-1, 101-1, 101-2, 101-3, and 102-1). All other fetuses were stimulated with current from a gas discharge valve (thyatron) delivering variable strength condenser shocks ranging in frequency from one shock to 460 per sec. Current strength ranged from 1 to 20 v. Resistances were not determined. Duration of shock (Fig 1) was approximately 70 μ sec. Galvanic current was tried but discarded because of the intense polarization and ensuing damage to the fetus.

Nearly all specimens were subjected to some form of mechanical stimulation, such as with a fine hair or by gentle prodding or displacement of parts, but these procedures did not result in visible muscular contraction except in one specimen, described below.

PHYSIOLOGICAL OBSERVATIONS ON RESPONSIVE
SIXTEEN-DAY FETUSES

(1) *Types of forelimb movements.* In 2 of the 16-day fetuses, 19-1 and 25-2, only shoulder muscles were visibly reactive to electrical stimulation. All other responsive specimens, however, produced more extensive and varied movements, involving shoulder, elbow, and wrist, separately or in several combinations. There was considerable individual variability. Finger

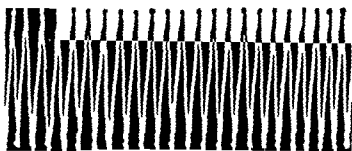


FIG. 1. Form of thyatron shock (above), and a frequency of 10,000 per sec. (below) for comparison.

movements never were observed. Stimulation of points over the shoulder or in the upper arm often elicited extensive movements, such as abduction of the entire limb accompanied or followed by dorsiflexion (hyperextension) of the wrist. Elbow extension sometimes was included in this movement. Dorsiflexion of the wrist was very easy to produce, this in contrast with movements of the elbow-joint. In 20-3, however, stimulation in the region over the brachial plexus gave rise to a striking diphasic displacement of the elbow, consisting of extension followed by flexion; occasionally this appeared with phases reversed, flexion then preceding extension. In general, the extensor musculature appeared more responsive than the flexor; but this may have been more apparent than real, for the position of the limb at this age is such that the extensors are more easily subjected to thorough experimentation.

(2) *Character of movements.* The forelimb movements of 16-day rat fetuses characteristically were of relatively long duration so that, in contrast with the adult, the moving parts travelled quite slowly. Both phases of the movement—active (muscle shortening) and passive (muscle relaxation)—were prolonged, the latter phase appearing to be especially lengthened in most instances. Repeated stimulation tended to lengthen both phases, as with 20-1, in which the movements became perceptibly slower after 8 or 10 responses. There was no indication, however, that the contractions were maintained when a current of liminal duration was applied. The response, therefore, is distinguished by marked prolongation of both phases of the movement rather than by anything suggesting a tonic state of muscular contraction. This typical slow movement was elicited by both methods of stimulation—faradic current and condenser.

Fetus 20-1 was studied to determine the effects of prolonged stimulation. Condenser shocks at a frequency of about 100 per sec. were employed. The

electrodes were kept in contact with a point on the dorsum of the upper arm for at least 10 sec. Not only were both elbow and wrist extended, but they were so maintained in what resembled a tetanic or tonic contraction for the duration of the stimulus. Ensuing responses to stimulation were feeble. That this particular reaction may have been tetanic, is suggested by the response of this same fetus to similar treatment with shocks given at a frequency of 1 per sec. These produced a relatively feeble, clonic tremor.

Fetuses 25-2 and (some movements only) 20-3 were the only specimens in which rapid movements were noted.

The muscles in many fetuses seemed to fatigue rapidly, visible reactions sometimes ceasing after a few responses to stimulation. But their apparently complete recovery following 5 or more minutes of rest in some instances is a proof that this was not the result of injury. Fetus 19-1 gave only one visible response to stimulation, although the experiment was continued for over one-half hour. In all other reactive fetuses, however, the movements were capable of reproduction. Two of the fetuses of rat no. 20 and the three of rat no. 101 differed from the other specimens in that they exhibited no signs of especially early fatigue. These variations appear incapable of explanation at the present time.

(3) *Strength of current.* The threshold of visible contraction in the fetus usually was quite high when contrasted with that of the mother, the maternal m. rectus abdominis usually being employed as a basis for comparison. In experiments where the faradic current was used, movements never were elicited if the secondary coil of the inductorium was withdrawn more than 4 cm. from the primary coil (with a current of 2 V on the latter). This was a much stronger current than that required to elicit contraction of adult rat muscle.

The same occurred, but with some individual variability, when using the condenser shocks. With these, from 7 to 10 V regularly caused contraction of the maternal rectus at a frequency of 100 or less per sec. A much stronger stimulus, ranging from 13 to 20 V, was needed to procure movement in the fetuses. As little as 7-10 V, however, at first produced some visible response in 20-1, but the general threshold soon was greatly elevated.

(4) *Time factor.* In most instances—regardless of the type of current—there was an appreciable interval between the application of the stimulus and the perceptible beginning of a movement. At times this exceeded a second. Indeed, in some instances visible responses could be obtained only after continued or repeated stimulation.

Condenser discharges much below a frequency of 100 per sec. were mostly ineffective in producing movements in the fetuses, although effective in adults. Fetus 20-1 was a notable exception, for, as mentioned above, it showed weak, clonic movements with shocks given at a frequency of one per sec. In addition, with shocks of 100 per sec. this specimen exhibited no appreciable delay of response until a series of rapidly sequential movements was induced. On the other hand, 19-2 failed to respond at a frequency of less

than 150 per sec. High frequency in itself, however, was inadequate if the voltage was too low.

(5) *Excitation through nerves.* In at least 5 of the fetuses it was possible to produce contraction of more distal muscles by electrical stimuli applied to proximal parts of the limb. For example, in some specimens stimulation of certain well-defined points on the dorsum of the upper extremity well above the elbow gave rise to dorsiflexion of the wrist. This was accompanied or preceded by abduction of the shoulder (and in certain instances extension of the elbow), or even could occur as an isolated movement. The possibility of spread seems to be excluded by the fact that stimulation of closely adjacent points caused no response. Return to the original excitable area invariably duplicated the movement. The most reasonable interpretation of this phenomenon is that we are dealing with excitation of muscles through their nerves, and that the excitable areas represent "motor points."

Four fetuses (10-2, 11-1, 19-1, 101-1) were stimulated in the region of the brachial plexus by introducing the electrodes through a skin incision. Three of these experiments produced no visible muscular contraction. In the fourth (10-2), however, typical movements, including the wrist, were obtained. Similarly, electrical stimulation of the skin over the brachial plexus area in 20-3, without insertion of the electrodes, gave rise to contractions of distal forelimb muscles. The movements seemed to vary with the points stimulated. The same results were obtained by pressure applied to this region with a blunt object, but gentle stroking of the skin (touch?) was ineffective. The negative results should not be stressed in view of the technical difficulties involved, but in the positive cases it seems clear that we must be dealing with effective conduction by efferent nerves.

In some instances, indeed, it appeared as if movements were more readily secured via the nerves than by direct stimulation of muscles. For, at times, distant stimulation proved successful when direct stimulation failed.

EXPLANATION OF FIGS. 2-6

FIG. 2. Section through superficial forearm extensors of 16-day rat fetus (13 mm C. R. length), showing myofibrillae in various stages of development, A, homogeneous fibrillae. B, beaded fibrillae. C, definitive fibrillae. Fixed in formalin-Zenker's fluid, 5 μ iron haematoxylin. $\times 1400$.

FIG. 3. Section through shoulder-joint of 16-day rat fetus (13 mm. C. R. length). Space between scapula (S) and humerus (H) occupied by mesenchyme; joint-cavity undeveloped. Fixed in formalin-Zenker's fluid, 5 μ , iron haematoxylin. $\times 90$.

FIG. 4. Section through shoulder muscle of 16-day rat fetus (13 mm. C. R. length), showing fine terminal nerve-fibers at X, slightly retouched. Fixed in 10 per cent formalin, 10 μ , Bodian's protargol stain. $\times 1750$.

FIG. 5. Section through forearm flexors of 16-day rat fetus (13.5 mm. C. R. length), showing nerve-fiber ending as a bulb at X. Fixed in 20 per cent formalin, 10 μ , Cuajunco-Bielschowsky silver stain. $\times 1750$.

FIG. 6. Section through long head of triceps brachii of 16-day rat fetus (13.5 mm. C. R. length). Nerve-fiber coming from above (not visible here) forms a bulb containing neurofibrillar net and continues as a fine, lightly staining, terminal filament (X). Fixed in 20 per cent formalin, 10 μ , Cuajunco-Bielschowsky silver stain. $\times 1750$.



FIGS. 2-6 (For legend see opposite page.)

HISTOLOGICAL STUDY OF THE FORELIMB IN
SIXTEEN-DAY FETUSES

The specimens are being studied histologically by one of us (W. L. S.). This investigation has not yet been completed, but it has revealed that the muscle-fibers and other structures within the forelimb of the 16-day rat fetus are morphologically immature.

The walls of the muscle-fibers are extremely thin. The nuclei are prominent and tend to occupy almost the whole width of the fiber. Myofibrils are relatively scarce. They sometimes occur as apparently homogeneous threads or beaded structures. Frequently, however, they exhibit high differentiation, with A and I segments, and in many of these a Hensen's stripe and a Z line also are visible (Fig. 2). Yet in such instances the fibrils are few in number, at times only four or even less to a fiber, so that definitive cross-striation of the fiber as a whole is not yet present.

Muscular fascia is just making its appearance; for, with Mallory's triple stain, little connective tissue is visible. Tendons have not been formed.

The joints are in an early stage of formation. The space between the skeletal elements is occupied by a rather dense mass of mesenchyme, so that articular cavities are not developed (Fig. 3).

Following silver impregnation, numerous primitive nerve-endings can be seen within the forelimb muscles but no motor end-plates such as are present in the adult. These endings are extracellular; they have not been seen within the cytoplasm of the muscle-fiber. Sometimes they appear to lie between the individual muscle-fibers rather than upon them. They assume a limited variety of forms. Most of the fibers terminate as slender threads that seem to fade out beyond the limits of microscopic visibility (Fig. 4). Possibly, however, this merely represents lack of argyrophilia at their ends. In other instances the nerve-fibers end as solid bulbs or loops that sometimes contain neurofibrillar nets; or they possess enlargements that distinctly resemble the growth cones described by other workers, as Speidel 52 (Fig. 5, 6). It is not certain that any of these structures are definitive nerve-endings. They all may represent areas of fiber growth.

DISCUSSION

The visible contractions of forelimb muscles in 16-day-old rat fetuses, induced by electrical stimuli, are characterized by (i) relatively long duration of the movement, involving both its active (muscle shortening) and, especially, passive (muscle lengthening) phases; (ii) comparatively high threshold of excitability; and (iii) relatively long delay of visible response. These properties can be stated only in general terms at this time because of the difficulties of applying more precise physiological procedure to specimens of this age. The deviations in certain features of the response exhibited by some specimens we are unable satisfactorily to explain. Their significance must await further study. These observations confirm and supplement those

secured by Windle *et al.* (30) from direct stimulation of rat fetuses, although these authors noted neither appreciable time factor nor tendency toward early fatigue.

The most obvious interpretation of our results would lead to the assumption that the visible characteristics of the induced movements accurately reflect the intrinsic physiological properties of the muscles involved. If such reasoning be acceptable, our findings indicate that slow contraction, high threshold and long time factor all are common to immature skeletal muscle, as in these 16-day rat fetuses. The long time factor can be interpreted as probably involving both a long latent period and a long muscle chronaxie. The former is suggested by the delay of visible response following stimulation. A long chronaxie would seem to explain our results following stimulation at various frequencies of condenser discharges. The necessity for continued or repeated stimulation, and for relatively high frequency in the case of the condenser—where duration of shock was short—both suggest the phenomenon of summation or latent addition. That this is characteristic of tissues with long chronaxie, is well known (13).

The above conclusion would agree fundamentally with the findings of other workers. Thus the electrically induced contractions of mammalian striated muscle have been found to be slow or of long duration in both young fetuses (4, 30) and older fetuses or new-born or very young animals (10, 15, 20, 22, 24, 28). A marked retardation of relaxation is emphasized by most of these investigators. Contractions likewise are slow in the chick embryo (18) and small amphibian larvae (6, 9, 26, 31). A high threshold of excitability, on both direct and indirect stimulation, often has been noted in fetal and new-born mammalian striated muscle. This is true for both the galvanic (15, 27, 28) and faradic (4, 15, 24, 27, 28, 30) forms of current. Conditions are similar in the chick (18). Some workers have reported a distinct or long latent period for the muscles of fetal or new-born mammals (1, 2, 4, 10, 20) and for chick embryos (17). Furthermore, it has been found that young mammalian skeletal muscle exhibits an exceedingly long chronaxie when compared with adult muscle. The data on this point have been summarized by Quincke and Stein (21). The chronaxie of the 5-day chick embryo also is long, being 60 times that of the adult fowl (16). These results might be expected since there is a direct relationship between length of chronaxie and duration of contraction (14). A tendency toward early fatigue of skeletal muscle by electrical stimulation was noted in our rat fetuses, and has been found in chick embryos (16, 18). Whether this depends upon factors intrinsic to young muscle or upon more general physiological conditions, can not now be decided.

It may be noted that immature skeletal muscle appears to be possessed of certain properties revealed by normal adult striated muscle only in electrically induced reversible contracture: Excessive duration of contraction and long excitation time (long chronaxie) especially as expressed by the

necessity for latent addition or summation (5). In many respects the visible reactions bear a curious resemblance to the Vulpian-Heidenhain phenomenon as summarized by Gasser (8).

Rückert (22, 23) concludes that the "tonic" or contracture-like capacities of a skeletal muscle stand inversely to its ontogenetic and phylogenetic age. In other words, the slow form of contraction is primitive and the rapid twitch advanced. Our observations on rat fetuses appear to support this thesis in so far as individual development is concerned: for the relatively long duration of the movements seemingly point to slow muscular contraction as its basis. It was noted that the muscle-fibers of the 16-day rat embryo differed from those of the adult, being morphologically immature. It would not be surprising, therefore, if the fetal muscle differed physiologically as well.

Not only the muscle, but the entire forelimb of the 16-day rat embryo is extremely immature. To assume that the visible responses accurately portray the physiological properties of the muscles concerned, may accordingly be inadmissible. Muscular fascia is quite rudimentary, while tendons are not yet developed. These are factors that readily may affect the efficient utilization of the muscular contraction. Furthermore, the articulations are in the earliest stages of formation and there are as yet no joint cavities. The limb therefore has the appearance of a relatively rigid structure and the muscles likely are operating upon comparatively stiff levers. In totality, these factors—which may be termed extramuscular—well may influence the nature of the response and produce a visible movement that fails to reflect the true character of the muscular contraction itself. It is entirely conceivable that the limb segments at this early stage may be possessed of considerable inertia relative to the force of contraction produced. It is quite possible that this would influence apparent time factor and duration of movement. Hence the seemingly long latent period, long chronaxie, and slow contraction all may be false phenomena resulting from the modifying effects of the peculiar extramuscular conditions.* Indeed, in view of the necessary experimental conditions, this possibility cannot be excluded. The small size of the fetuses precludes the usual experimental treatment of the muscular tissue, so that comparison with results obtained from adults or even new-born or late fetal stages seems unjustifiable. The intrinsic physiological properties, under normal experimental conditions, of young skeletal muscle—as in the 16-day rat embryo—therefore as yet are unknown.

Our experiments have produced evidence that potential neuromuscular conduction, at least on the efferent side, already is established in rat fetuses in the first half of the 16th day of gestation. Primitive nerve-terminations are present within the muscles of the forelimb, but it is extremely unlikely

* The possible effect of the surrounding fluid on the response likewise should be taken into account. It cannot be estimated, but apparently it exerts some buoyant effect that conceivably may modify the duration of a movement: for when the fetuses are removed from the bath they tend to flatten out, while the limbs so adhere to the trunk that visible somatic responses to stimulation cannot be elicited.

that any definitive nerve-endings have as yet been formed. At this stage, then, there exists the capacity for effective neuromuscular transmission in the absence of intimate connection between striated muscle and nerve. One therefore cannot ignore the possible rôle of nerves even in direct stimulation of the muscles in these young fetuses. It would seem desirable, moreover, to discard the term "myogenic" used by some authors to denote the earliest visible contractions that follow direct stimulation of fetal mammalian striated muscle (1, 2, 3, 30). This term is misleading, since no unequivocal proof of a purely "myogenic" stage of activity of striated muscle in mammals, as contrasted with "neurogenic," exists. Kuo (11) takes a similar stand. His conclusion (12), based on the effects of curare, that all somatic movements in young chick embryos are nervous in origin and that the skeletal muscles are incapable of activity without functional nerves, appears unconvincing, however; for he has assumed, without adequate evidence, that the reactions of embryonic and adult tissues to the drug are identical. Paton (19) observed somatic movements in embryos of the selachian *Pristiurus melanostomus* prior to innervation of the skeletal musculature as judged by the presence of neurofibrils following silver impregnation; but since he found "undifferentiated protoplasmic bridges" between spinal cord and myotome, Paton left the nature of the movements, whether myogenic or neurogenic, an open question. Possibly conditions differ among the various classes of vertebrates, but as yet there is no conclusive evidence that striated muscle ever includes an aneural active phase in its normal development.

It was noted that in some cases the forelimb muscles of 16-day-old rat fetuses appeared more readily excitable through their nerves than by direct stimulation. Our findings possibly imply a difference in chronaxies of muscle and nerve in young fetuses; or they merely mean that a direct stimulus sometimes may be too localized to produce more than a feeble, imperceptible contraction, whereas indirect stimulation, exciting a large number of nerve-fibers, provokes a more widespread, visible reaction.

A discussion of prenatal reflex activity lies outside the scope of this paper. But the possibility that some of the muscular responses in fetuses of this age are true reflexes, cannot be ignored. Since the dorsal spinal roots are well-developed, some of the nerve fibers within the muscles may well be of an afferent type. Windle and Baxter (29) found that morphologically complete reflex arcs already exist at this time. All of our results, however, appear capable of explanation with reference to efferent nerves alone, and we have observed nothing that can be termed reflex activity without question.

SUMMARY

The earliest visible contractions of skeletal muscle in the living, intact rat fetus were produced by electrical stimulation during the 16th day of gestation. These involved a variety of forelimb movements.

The movements typically exhibited high threshold, relatively long duration (especially of the phase of relaxation), tendency toward rapid fatigue,

and long time factor (suggestive of both long latent period and long chronaxie of the muscles).

The limb at this stage is in a state of early differentiation, including not only muscle-fibers but also fascia, tendons and joints. This strongly suggests that extramuscular factors may be influencing the nature of the muscular response, whose visible properties thus may be more apparent than real. It is concluded, therefore, that the somatic movements cannot be unequivocally accepted as reflecting the physiological capacities of the muscles involved.

Evidence is presented indicating that potentially effective neuromuscular transmission, on the efferent side at least, exists in 16-day rat fetuses, although the nerve-endings themselves are primitive in form.

The theory of a "myogenic" developmental phase of muscular activity is discussed, and it is concluded that no valid evidence for such a phase exists.

This communication is based chiefly on work done at University College, London. We are greatly indebted to the late Professor H. H. Woollard, F. R. S., for the facilities of his laboratory and for his great interest in, and encouragement of, this work.

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ACTIVITY IN THE SIMPLEST SPINAL REFLEX PATHWAYS

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ALTHOUGH Cajal (1890, 1894) demonstrated that collaterals of the dorsal root fibers extend through the cord to the anterior horn, there to make synaptic connections with motoneurons, and although reflex arcs of two neurons, *i.e.*, of one synaptic relay, have played a prominent role in schemata of cord activity (*cf.* Cajal, 1909), there has been little physiological evidence of activity in such direct pathways.

Most of the determinations of the central reflex time for homolateral responses in the cord have given minimal values of approximately 3 to 5 msec. (Jolly, 1911; Forbes and Gregg, 1915; Eccles and Sherrington, 1931a). Eccles and Sherrington, however, found that these values, determined by recording the activity of a flexor muscle, may be greatly reduced when the cord is conditioned by a previous volley arriving over the dorsal roots; and Eccles and Pritchard (1937), by applying strong electrical stimuli to dorsal roots, obtained from the unconditioned cord ventral root discharges with reduced latencies of 0.7 to 1.0 msec. Since, according to Lorente de N6 (1935a, c, d; 1938a), the synaptic delay at the motoneurons of the third cranial nucleus varies within the narrow limits of 0.5–0.6 to 0.8–0.9 msec., and since comparable delays have been observed in other pathways (Kemp, Coppée and Robinson, 1937; Bishop and O'Leary, 1938), it is likely, as Eccles (1939) points out, that these minimal central reflex times for the cord pertain to two-neuron pathways.

In the present work determinations of the synaptic delays at neurons in the spinal cord, in conjunction with measurements of central reflex times, demonstrate the validity of this interpretation. Under certain experimental conditions activity in arcs of two neurons is a prominent feature of cord activity. The facilitation and inhibition of activity in these direct pathways have been examined.

METHODS

Cats, either decerebrated or under light Dial anesthesia (Ciba, 0.4–0.6 cc/kg) were used. The spinal cord was often transected at the lowest thoracic or the highest lumbar level. One or both dorsal roots and the ventral root on one side of either the seventh lumbar or the first sacral segment were prepared. Pairs of stimulating electrodes were applied to the dorsal roots. The proximal electrode in each case was 12 to 15 mm from the cord. A second method of stimulation was through bipolar needle electrodes inserted into the gray matter. The discharge in ventral roots was led from electrodes placed sufficiently far from the cord to prevent appreciable complication of the record by the "electrotonic" ventral root potentials of Barron and Matthews (1936, 1938). The potential changes de-

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† A preliminary report of these experiments was made at the meeting of the American Physiological Society, New Orleans, La., March 15, 1940.

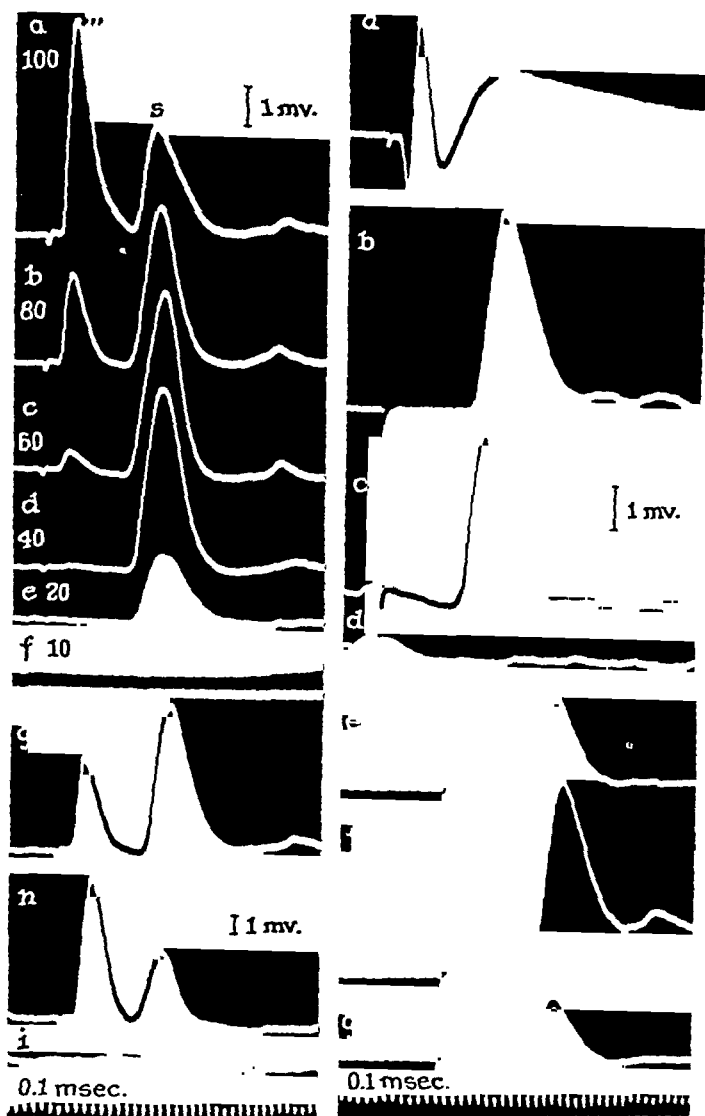


FIG. 1.(left). Ventral root discharge following stimulation by needle electrodes in the region of the intermediate gray matter. Decerebrated cat acutely spinal at the highest lumbar level. *a-f*, decreasing stimulus strengths, relative values indicated on the figure. *g-i*, the effect of conditioning the cord by a maximal dorsal root volley; *g*, needle stimulation; *h*, needle stimulation following a dorsal root volley by 3 msec.; *i*, end of response to dorsal root stimulation. Time, 0.1 msec.

FIG. 2 (right). *a*, cord potential, monopolar lead from the dorsum of the cord, stimulus a shock maximal for A fibers applied to the dorsal root. *b-g*, discharge in ventral root following stimulation of dorsal root. Same preparation as Fig. 1. *b*, discharge due to single maximal A volley. *c*, the same conditioned by a similar volley preceding by 3 msec. *d*, end of the response to the conditioning volley alone, showing baseline on which the response to the testing volley wrote. *e-f*, effect of DC polarization of the cord; current passed between the ventral root and muscles on the opposite side at the same level of the vertebral column. *e*, no current; *f*, 20 μ A, root positive; *g*, 20 μ A, root negative. Time, 0.1 msec.

veloped in the cord were recorded between an indifferently placed electrode and a small Ag-AgCl ball, 0.5 to 1.0 mm. in diameter, placed on the dorsum of the cord. For the determination of activity at points within the cord, the ball electrode was replaced by a micro-electrode which could be inserted into any desired position; use was made both of micropipettes and of fine steel needles insulated except near the tip. The customary stimulating apparatus and differential amplifier were used. The preparations were covered with paraffin oil to a depth of about one centimeter, in order to help maintain the cord and its roots at the proper temperature (*cf.* Table I) and otherwise in good condition for long periods of time.

RESULTS

1. *Synaptic delay at motoneurons.* The synaptic delay at the motoneurons in the cord has been determined by a method originally used for another system by Lorente de N6 (1935d, 1939). Small bipolar needle electrodes were inserted into the cord, in order both to stimulate some motoneurons directly and to activate fibers and collaterals making synaptic connections with additional motor cells. The discharge in an adjacent, homolateral ventral root following such stimulation produces the two prominent waves that have been labelled *m* and *s* in Fig. 1 and 3. The threshold of stimulation for the *m* wave is lower than that for the *s* wave when the stimulating electrodes are in ventral positions within the cord—at or below the ventral horn. It is lower for the *s* wave when the electrodes are located more dorsally. The *m* wave represents a practically synchronous volley of impulses in fibers of the ventral root, and its latency is approximately accounted for by conduction from the cord to the recording electrodes. It therefore arises from direct electrical stimulation of the motoneurons.

The *m* wave may be conditioned by the delivery of a previous shock to the dorsal root (Fig. 1*g-i*, 3). Since in the experiment from which the records of Fig. 1*g-i* are taken some motoneurons had responded to the conditioning shock, it might perhaps be thought that the increase in the *m* wave in record *h* is attributable to direct stimulation by the testing shock of additional motor fibers which were in a supernormal condition. This is scarcely possible, because the first motoneurons responded to the conditioning volley only 2.2 msec. (shock interval, 3.0 msec.) before the application of the test stimulus causing the *m* wave; and according to Gasser and Grundfest (1936) and Graham and

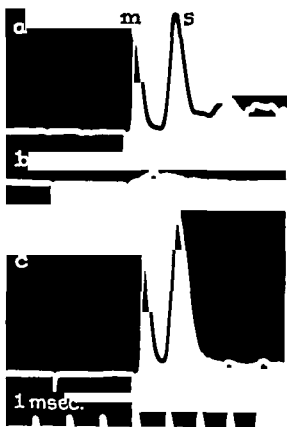


FIG. 3. Ventral root discharge following stimulation of the cord through needles in the central gray matter. Decerebrated cat, acutely spinal at the highest lumbar level. *a*, response to needle stimulation; *b*, to submaximal stimulation of the homolateral dorsal root; *c*, needle and root stimulation in combination. Time, 1 msec.

Lorente de Nó (1938) refractoriness gives way to supernormality in blood-perfused nerves only after 2 to 5 msec. Moreover, in the experiment of Fig. 3 facilitation of the *m* wave was observed without previous reflex discharge. Therefore, the facilitation of the *m* wave must be interpreted as due to a summation of the induction shock with subliminal synaptic stimuli (Lorente de Nó, 1935d; Lorente de Nó and Graham, 1938).

The *s* wave, which represents a somewhat asynchronous volley of impulses, follows the *m* at an interval of about 0.7 to 0.8 msec. As the stimulus is increased in strength from low values, both waves increase in size together until at high values the *s* wave decreases as the *m* wave continues to grow (Fig. 1*f-a*), obviously because motoneurons responding in the *s* group have

Table 1. Central reflex times and synaptic delays at motoneurons, determined together in several preparations

Experiment	Preparation	Rectal temperature	Cord temperature (Thermocouple)	Synaptic delay at motoneurons (msec.)		Central reflex time (msec.)	
				Unconditioned	Conditioned	Unconditioned	Conditioned
5/23/39	Decerebrated	38.0 — 38.4		0.7+	0.6	0.8–0.85	0.6–0.7
6/17/39	Decerebrated	36.8 — 37.8		0.9	0.65	0.9	0.7
6/27/39	Decerebrated	40.2 — 40.5	39.7 ± 0.2	0.8 —	0.6 —	0.65	0.5+
8/ 7/39	Decerebrated	40.0		0.9	0.7	0.85	0.7
10/10/39	Spinal	38.5		0.75	0.7	0.65	0.5

shifted to the *m* and become refractory. It is to be emphasized that the reduction of latency when motoneurons responding in *s* pass into *m* is not gradual but discontinuous. The interval between the two waves remains nearly constant over a considerable range of stimulus strengths and cannot be decreased by more than 0.1 to 0.3 msec., even when the cord has been conditioned by a maximal volley in the dorsal root (Fig. 1*g-i*). As a rule, the intervals between the feet of the *m* and *s* waves have ranged from 0.6 to about 1.0 msec. Since the conduction time for impulses exciting the motoneurons responding in the *s* wave must be small and since no procedures that have been tested reduce this interval significantly, it has been interpreted as representing the duration of one synaptic delay, i.e., that at the motoneurons. The values obtained in several experiments are presented in Table 1.

2. *Central reflex time for homolateral reflex.* A close parallel exists between the results reported in the preceding section and observations made on central reflex times (see Table 1). The discharge in a ventral root, due to a volley maximal for most of the A fibers other than the delta group in the cor-

responding homolateral dorsal root, is usually characterized by an early wave (Eccles and Pritchard, 1937), with an unreduced latency of 1.0 to 1.4 msec. (Fig. 2 and 4). The well-known discharges which last 8 to 10 msec. or more often follow. The first wave may be small compared with the later phases, but usually it is the conspicuous deflection of the reflex oscillogram. The size of the intramedullary spike of the cord potential may be used as a rough measure of the number of A fibers stimulated. Judging on this basis, the appearance of the early wave generally requires the stimulation of $\frac{1}{4}$ to $\frac{1}{2}$ of the A fibers of the dorsal root. With weaker stimuli, responses of longer latency appear, and by varying the strengths of the testing and conditioning shocks applied to the dorsal roots, a continuous series of reflex latencies, ranging upward from the minimum, may be obtained.

The central reflex time is the observed reflex latency, minus conduction time in the afferent and efferent nerves. Conduction time in the dorsal roots has been measured as the interval between the stimulus escape and, as a good approximation to the time of arrival of impulses at the cord, the time corresponding to half the descent into the first positive trough of the primary spike of the cord potential (Fig. 2*a*). The latency of the *m* wave in such records as *a-c* of Fig. 1 has been taken as the conduction time in the ventral root. Since this correction is small and little altered by a considerable increase in the strength of the stimulus (Fig. 1*c-a*), the use of the shock-response interval for the determination of conduction time is justified. Except for about 3 mm. of the relatively stout "col-latérales réflexo-motrices" (Cajal, 1909),

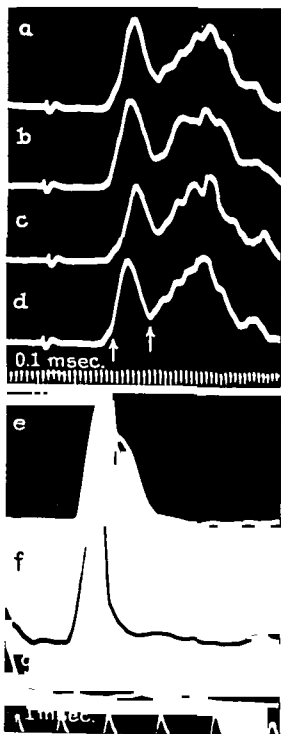


FIG. 4. Reflex discharges in ventral roots showing discontinuities in the populations of responding motoneurons. Discharges in ventral roots following the delivery of a single shock, maximal for A fibers, to the corresponding homolateral dorsal roots, *a-d*, decerebrated cat, acutely spinal at highest lumbar level. The corrections for conduction in the roots amounted to 0.4 msec. Discontinuities in the discharge occur at central reflex times of about 1.0 and 1.7 msec. Time, 0.1 msec. *e-g*, decerebrated cat. The corrections for conduction time in the roots amounted to 0.4 + msec. *e*, single testing volley; *f*, the same preceded by a similar volley; *g*, end of the response to the conditioning volley alone. Note the discontinuity in *e* at a central reflex time of about 1.7 msec. and its absence in *f*. Time, 1 msec.

which extend from the dorsum of the cord to the anterior horn, these corrections completely account for conduction time in the fast fibers of the direct reflex pathways.

In the experiment from which the records of Fig. 1 and 2 have been taken the corrections for root conduction are each about 0.2 msec. (Fig. 2*a*; 1*a-c*). The uncorrected latency of the first portion of the early wave of the discharge from the unconditioned cord is 1.05 msec. (Fig. 2*b*), giving a

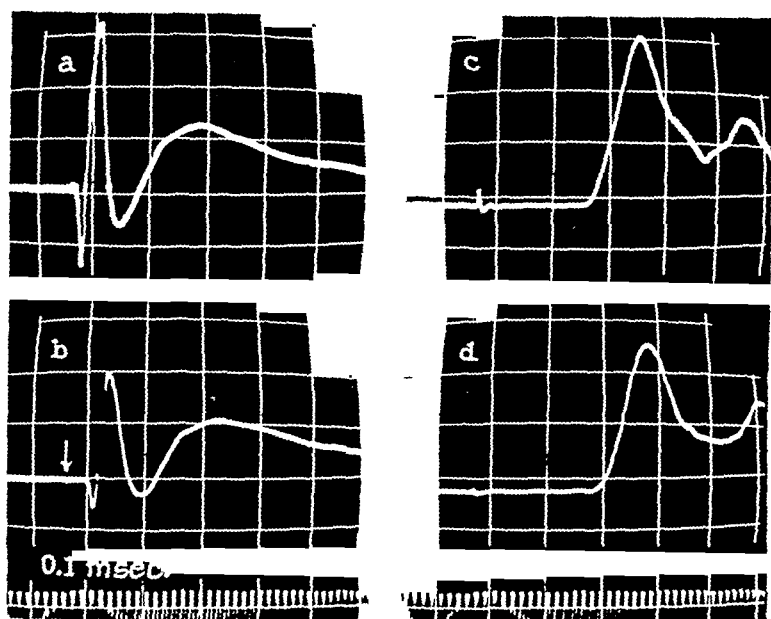


FIG. 5. *a, b*, cord potentials recorded from the dorsum of the cord; *c, d*, the corresponding reflex discharges in the ventral root. *a* and *c*, are responses to shocks applied to the dorsal root a few mm. from the cord; *b* and *d* are responses to stimuli applied 17 mm. more distally. All the shocks were 5 times the threshold for the intramedullary spike and the negative cord potential. Time, 0.1 msec.

central reflex time of 0.65 msec. As shown in Fig. 2*c*, this was reduced to about 0.5 msec. by a maximal conditioning volley preceding the testing in the same dorsal root by 3.8 msec. The data of several experiments are presented in Table 1.

The corrections for conduction time in the dorsal roots were based upon activity of the fastest fibers. The dorsal roots contain A fibers of a wide range of conduction velocities (Gasser and Grundfest, 1939), and the central reflex times as determined would be in error if activity in fibers conducting at significantly less than the maximum velocity were important for the reflex discharges. This possibility was tested in the following way (Fig. 5). Two pairs of stimulating electrodes were placed on a dorsal root, one pair near the cord and the other more distally. The distance between cathodes was 17

mm. The reflex discharges in the corresponding ventral root, and the potential changes at the dorsum of the cord, were recorded for graded series of stimuli applied through each pair of electrodes.

The shock-response intervals for various points on the first portion of the intramedullary spike which followed distal stimulation were greater than the corresponding intervals for proximal stimulation (Fig. 5a, b). The difference indicated a maximal conduction velocity in the dorsal root of about 80 m. per sec., which approximates the known value for the fastest sensory fibers. The latency of each point of the early part of the reflex discharge to stimulation via the distal electrodes was also greater than that to stimulation through the proximal electrodes (Fig. 5c, d), and by an interval equal to or only slightly longer than the conduction time between the cathodes for the fastest afferent fibers. If activity in fibers of significantly submaximal velocity were important for the earliest discharges, then parts or all of the first wave of the reflex to distal stimulation would have appeared only after a greater delay.

In view of these data it is conservative to state that the first portions of the reflex discharge are due to activity in A fibers conducting at velocities ranging between maximal and no less than two-thirds maximal. The corrections for conduction time in the dorsal roots, based upon activity in the fastest fibers, amounted to about 0.2 msec. For fibers conducting at two-thirds the maximal velocities the corrections are increased by only 0.1 msec. Accordingly, the corrections as used cannot be in serious error.

These observations incidentally provide conclusive evidence that the stimuli applied to the dorsal roots did not spread to cause direct excitation of central structures.

An attempt further to shorten the central reflex time by polarization of the cord with constant currents, has failed. Barron and Matthews (1936, 1938) and Matthews (1937) have shown that the passage of small currents from a motor root to an adjacent point on the spinal cord causes a rhythmic discharge in fibers of the ventral root at frequencies up to 70 per sec., depending upon the strength of the current. No discharge occurs when the current is passed in the opposite direction, i.e., cathode on root. Figure 2e-g demonstrates that in fact very small direct currents, too weak to cause appreciable rhythmic discharge, nevertheless condition the cord. Figure 2e shows a central reflex discharge recorded with no current flowing, 2f the augmented response obtained when a current of 20 microamperes was passing from a point on the ventral root to an electrode placed on muscles of the contralateral side at the same level of the vertebral column, and 2g the reduced response seen during the passage of 20 μ A in the opposite direction. A current of 10 μ A was sufficient to produce smaller but definite effects of the same nature. Although in Fig. 2f the augmentation of the response is considerable, no appreciable decrease in latency occurred.

3. *Conditioning of first wave of homolateral reflex by single contralateral volley.* The data of the previous sections have been interpreted as demon-

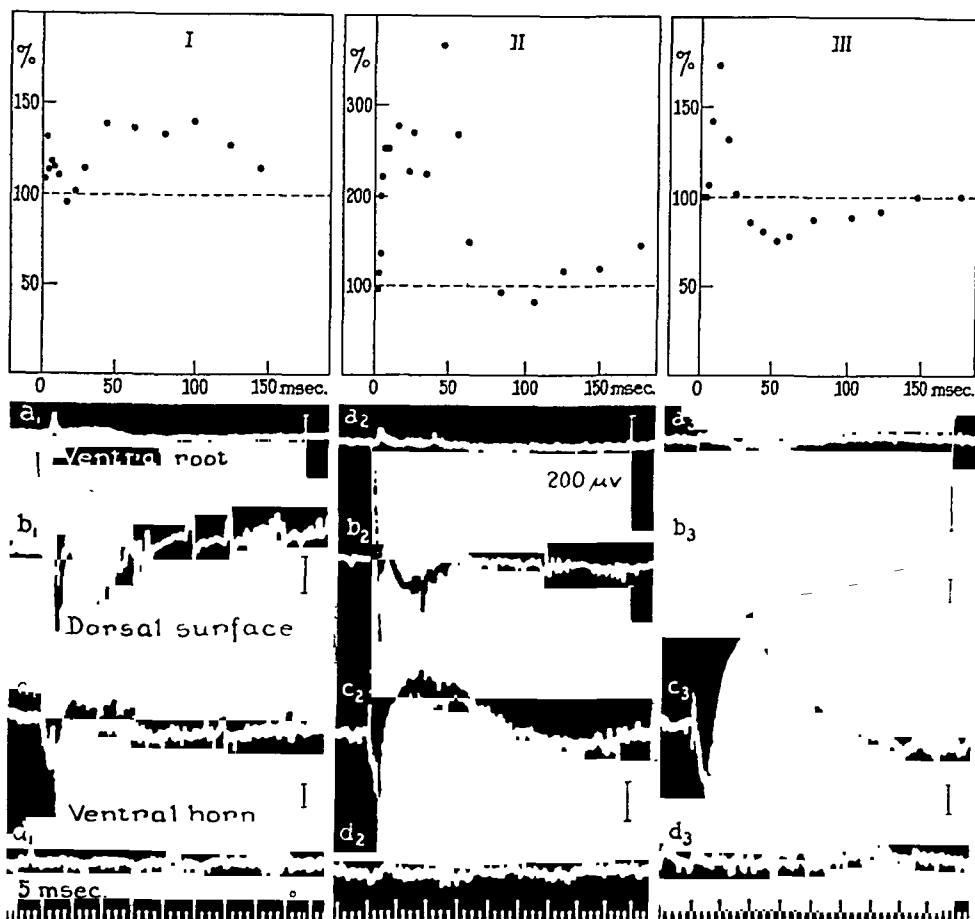


FIG. 6. Conditioning of the first wave of the dorsal-to-ventral root reflex by a single crossed volley. Data from three spinal cats, I and II decerebrate, III under light Dial narcosis. Abscissae of the graphs: intervals at which the homolateral stimulus followed the contralateral. Ordinates $\frac{\text{height of conditioned first wave}}{\text{height of unconditioned first wave}} \times 100$. The oscillograms show the responses to the crossed volley alone: a, reflex discharge in ventral root; b, potential changes recorded from the mid-dorsum of the cord; c, potential changes recorded by a micro-electrode in the ventral horn; d, as c but without the stimulus, to show the background of spontaneous activity. Voltage calibrations, 200 μ V. Time, 5 and 20 msec.

strating that the early wave of the reflex discharge is a measure of activity in arcs of two neurons (see Discussion). Consequently there becomes available a convenient method for testing the average synaptic excitability of the motoneurons in the pool supplied by the stimulated dorsal root fibers; for these motoneurons may be directly stimulated at any chosen moment by a controlled afferent volley which produces a measurable submaximal discharge.

The conditioning of the first wave of the homolaterally evoked reflex by a single contralateral volley has been examined in a series of 23 spinal cats. Figure 6 presents data obtained from three of these. In addition to the conditioning curves, the figure includes oscillograms to show that a small reflex discharge is evoked by the contralateral volley. Also included, as indices of the internuncial activity induced by the contralateral volley, are records of the cord potentials produced by it on the mid-dorsum of the cord and in the ventral horn.

The conspicuous effect of a single crossed volley on the reflex activity of a flexor muscle is typically inhibition (Samojloff and Kisseleff, 1927; Eccles and Sherrington, 1931b; Hughes, McCouch and Stewart, 1937). Under the conditions of the present experiments, however, the early reflex discharges in the ventral roots of the majority of active, decerebrated preparations are facilitated by the contralateral volley. The facilitation may amount to several hundred per cent and last for 150–200 msec., as in curves I and II of Fig. 6. Other preparations, including nearly all animals under light Dial narcosis, yield conditioning curves in which facilitation gives way after 10–25 msec. to a more prolonged period of inhibition (curve III). The maximal inhibition occurs 30–50 msec. after the contralateral stimulus; at this time the height of the first wave of the conditioned testing volley is reduced to 50–80 per cent of its unconditioned size.

DISCUSSION

The similarity of the values for the shortest central reflex time and the interval between the *m* and *s* waves (Table 1) demonstrates that the earliest reflex discharges represent activity in the direct pathways which have only a single synaptic relay; and the fact that it has not been possible to reduce either the central reflex time or the *m-s* interval below about 0.5 msec., suggests that this is a minimal value for the synaptic delay at the motoneurons.

The question that next arises, is, how long may be the synaptic delay? The central reflex times obtained for conditioned and unconditioned reflex responses to dorsal root volleys of varying size represent a series, continuous to the precision of the measurements, extending from 0.5 msec. to 2 msec. or more; and in many experiments in which the first motoneurons respond at the shortest central reflex times there is clear evidence that others become active at progressively longer intervals. The long latencies do not necessarily signify prolonged synaptic delays, however, and considerable evidence favors the alternative view that they represent activity in arcs containing interneurons (Lorente de Nó, 1938b). It is known that interneurons are present. They may be interpolated between the dorsal root fibers and the motoneurons to form reflex arcs of two, three and more synaptic relays; and they are activated by afferent impulses (Gasser and Graham, 1933; Hughes and Gasser, 1934a). A synaptic delay is properly measured from the time of arrival of the latest impulse which is effective in firing the post-synaptic

neuron. Hence, because of the probability that motoneurons are effectively stimulated by the delayed internuncial impulses, it is difficult to determine the maximal central reflex times associated with activity in the direct pathways. It may only be stated that the range of latencies must extend from 0.5 msec. upward to at least the minimal central reflex time for activity in three-neuron arcs. This shortest central reflex time for pathways of three neurons can be no less than the sum of the minimal delay at a motoneuron and the minimal delay at an interneuron. The duration of the delay at interneurons thus becomes a matter of interest.

The available evidence suggests that 0.5 msec. is an approximate minimal value for the delay at interneurons, as well as at motoneurons. Inspection of Fig. 2a, which represents the potential changes produced at the dorsum of the cord by an afferent volley, shows that the curve crosses the baseline to rise into the negative cord potential (Gasser and Graham, 1933) only 0.6 msec. after the impulses in the dorsal root fibers have reached the cord. This suggests that 0.6 msec. is an upper limit for the synaptic delay at the earliest responding interneurons. Stewart, Hughes and McCouch (1940) report slightly longer latencies in the monkey. Similar short delays have been found in other parts of the nervous system. Lorente de Nó (1939) reports that in the internuncial nuclei associated with the oculomotor nucleus the synaptic delays correspond to those at the motoneurons; that is, they may be as short as 0.5 to 0.6 msec. The results of Bishop and O'Leary (1938) indicate a delay of 0.5 msec. for visual impulses relayed at the lateral geniculate body; and Kemp, Coppée and Robinson (1937) have calculated the slightly longer minimal value of 0.8 msec. for impulses relayed in the auditory pathways at the cochlear nucleus and the olivary complex.

The sum of the shortest synaptic delays that have been determined for interneurons and motoneurons, each 0.5 msec., is 1.0 msec. Accordingly, unless internuncial delays of unsuspected brevity occur in the cord, 1.0 msec. must be the minimal central reflex time for activity in three-neuron arcs. Therefore, all central reflex times of from 0.5 msec. to 1.0 msec. must pertain to arcs of two neurons. With one reservation, the synaptic delays at the motoneurons must extend over this range of values. The reservation arises from the fact that the time for conduction in the 2-3 mm. of collateral within the cord was not considered; and, of course, the corrections for conduction time in the dorsal roots were based upon activity in fibers conducting at maximal velocities. The early part of the reflex is mediated only by fibers conducting at velocities near the maximal; the stoutness of the collaterals of these large sensory fibers (*cf.* Cajal, 1909) justifies the assumption that they do not conduct at low velocities. Consequently these factors can contribute at most an additional 0.1 msec. to conduction time, and 0.9 msec. becomes a conservative *minimal* value for the longest synaptic delay at motoneurons of the cord. At present it is not possible to state whether still longer delays occur.

Little is known of the range of variation of the delays at interneurons,

but if it be assumed that the interpolation of an interneuron into a reflex arc increases the central reflex time by an amount likewise varying between 0.5 and about 1.0 msec., then the central reflex times for three-neuron pathways can vary between 1.0 and 2.0 msec., for four-neuron pathways between 1.5 and 3.0 msec., and so forth. Motoneuron discharges, such as may be observed at all reflex times longer than the minimum, can therefore be accounted for. At the same time it becomes apparent that, except for the earliest discharges, the number of synaptic relays involved in the production of any reflex activity cannot be strictly calculated from the central delay. It is impossible, for example, to state whether motoneurons firing after a central time of 2.4 msec. represent activity in pathways of three synaptic relays with an average delay of 0.8 msec., or in arcs of an additional relay where the average delay amounts to only 0.6 msec., or in both.

Although some motoneurons may be fired at each moment during the period of reflex activity, the discharges do not give smooth curves. As reference to any actual record brings out, the synaptic delays gather about modes, and the motoneurons, especially in the early part of a discharge, tend to be fired in groups. Hence for the first portions of a discharge it is possible to make a fairly exact estimate of the number of synaptic relays involved.

The reflex discharges in the records of Fig. 4 exhibit typical grouping of the responding motoneurons and show the discontinuities which consequently appear at characteristic central reflex times. The discharges following four identical afferent volleys in a single experiment are shown in Fig. 4a-d. It is obvious that the grouping of responding units is relatively constant. At a central reflex time of 1.0 msec., as marked by the first arrow, the number of active units increases. Again at 1.7 msec., as indicated by the second arrow, the discharge is suddenly augmented. The second wave follows the first at an interval of 0.7-0.9 msec., the duration of an average synaptic delay. Figure 4e depicts the reflex discharge from another experiment. Activity begins after a central reflex time of 0.9 msec. and, as marked by the arrow, is also characterized by a discontinuity at about 1.7 msec. The activity following the discontinuity is largely abolished in the conditioned response shown in Fig. 4f, whereas the discharges at shorter reflex times behave as a homogeneous group and are somewhat increased.

In some experiments (Fig. 2b) the first wave of the reflex discharge is due almost entirely to activity of motoneurons which respond at central reflex times of 1.0 msec. and less. In the experiments of Fig. 4, however, the first wave represents the activity of motoneurons which fire at intervals ranging from 0.8 and 0.9 msec. to more than 1.0 msec., in fact to about 1.2 msec. The portion of the first wave which corresponds to central reflex times of 1.0 msec. and less must of course represent activity in arcs of two neurons. It cannot at present be stated with finality that the motoneurons which discharge in the later portion of the first wave have not been activated via an interpolated interneuron, rather than directly by the primary afferent fibers. At any rate, on conditioning the first wave behaves more or less as a

unit in facilitation and inhibition. Hence, if an interneuron is interposed, it must be one which follows the activity in the afferent fibers very nearly in a 1:1 ratio under various conditions. This hypothetical behavior is in contrast with that of the spinal interneurons which have been studied, for it is well known that these respond subnormally after a conditioning volley (Hughes and Gasser, 1934b).

The possibility of exciting motoneurons directly by a controlled afferent volley, the size of which does not vary with the level of excitation in labile internuncial systems, provides an excellent method for testing the average synaptic excitability of the motoneurons in the pool supplied by the stimulated dorsal root fibers. Illustrative experiments, in which the synaptic excitability of the motoneurons was tested at various intervals after the delivery of a contralateral volley to the cord, reveal the action of mechanisms for facilitation and inhibition which have been described by Gasser (1937a, b), Hughes and Gasser (1934b), and by Lorente de Nó (1935c, 1936, 1938b, 1939).

Although the contralateral volley fires few motoneurons, it facilitates others, and the direct response to a homolateral volley is increased for a period of time, as in all three curves of Fig. 6. In most decerebrated preparations the facilitation is long continued, as in curves I and II, with at most only transitory intervention of slight inhibition. In other experiments, particularly when a light dose of Dial is given, the facilitation passes over into inhibition after 10-25 msec., as in curve III.

The potential changes produced in the ventral horn by the crossed volley reveal a mechanism for the facilitation. Although the significance of the envelope to the sequence of potential changes is not yet clear, the width and roughness of the baseline may be used as indices of the level of background activity. Comparison of records *a* with *c* and *d* (Fig. 6) reveals that even in the ventral root lead, where recording is more favorable than in the volume of the cord, the active motoneurons produce relatively small deflections compared to the changes observed in the ventral horn. Consequently most of the background activity of the ventral horn must be interneuronal. At least some of the internuncial impulses must ordinarily impinge upon the motoneurons. The periods during which the testing two-neuron discharge is facilitated are characterized by an increase of the background of internuncial activity above its resting level (records *c*, also *b*). Facilitation, therefore, is accomplished by convergence of internuncial impulses with the primary testing impulses.

During inhibition (curve III) the deficit in the response of the two-neuron arcs must be due, at least in part, to subnormality in the few motoneurons which were fired by the contralateral volley. Antidromic stimulation has revealed that, following activity, motoneurons exhibit a prolonged period of lowered synaptic excitability (Eccles, 1931; Lorente de Nó, 1935b, 1939; Gasser, 1939).

Subsequent experiments have shown that, although in spinal animals most of the

unconditioned discharge of short central latency goes to flexor muscles some impulses are in fibers to extensors. Also in acutely spinal animals the contralaterally evoked impulses do not necessarily represent a discharge only of motoneurons associated with extensor muscles (McCouch, Snape and Stewart, 1935, McCouch 1936).

For the sake of completeness it may be mentioned that the conditioning volleys must have evoked crossed dorsal root reflexes. The centrifugal impulses of these discharges may have interfered with and blocked centripetal impulses of the testing volleys. Any such modification of large testing volleys must be slight, for crossed dorsal root reflexes are small and dispersed discharges (Toennies, 1938).

The cord potentials produced by the contralateral volley in the ventral horn point to the existence of a second factor for inhibition. Subnormality in interneurons may be expected to follow the increased activity which characterizes the antecedent period of facilitation, and, in fact, during the period in which the testing two-neuron discharge shows a pronounced deficit (curve III), the background of internuncial activity is decreased below the resting level (record c_3). Withdrawal of the facilitating effect of these impulses may raise the threshold of the motoneurons and aid in the production of the observed deficit.

SUMMARY

A shock which excites $\frac{1}{2}$ to $\frac{1}{3}$ of the fibers of maximal and nearly maximal conduction velocity in the seventh lumbar or the first sacral dorsal root of the decerebrated cat typically produces in the corresponding homolateral ventral root a reflex discharge with a central delay varying between 0.65 and about 1.0 msec. Conditioning the cord by means of a previous dorsal root volley may decrease the central reflex time slightly, but no procedures have reduced it below about 0.5 msec. Ventral root discharges resulting from direct electrical stimulation of the central gray matter demonstrate that the synaptic delays at the motoneurons vary over a similar range of values. Consequently central reflex times of 0.5 to about 1.0 msec represent activity in reflex arcs of two neurons (one synaptic relay). It is not possible to state whether or not longer synaptic delays sometimes occur, for central reflex times as short as about 1.0 msec may conceivably pertain to activity in arcs of three neurons.

Motoneurons may respond at any or all central reflex times for several milliseconds after the first discharge to a dorsal root volley—a result to be expected even if the synaptic delays at interneurons as well as at motoneurons range between limits no wider than 0.5 to 1.0 msec. Nevertheless, the synapse times show modes, and large groups of motoneurons are typically discharged at particular central reflex times.

Direct excitation of motoneurons by the primary sensory fibers offers a method of testing the synaptic excitability of the motoneurons. The motoneurons are facilitated for some time after the arrival at the cord of a volley over a contralateral dorsal root. During the period of facilitation, as can be determined from cord leads, the motoneurons are receiving a barrage of impulses from interneurons. Facilitation, therefore, is accomplished by convergence of internuncial impulses with the impulses of the testing volley. A

period of inhibition sometimes follows the facilitation. The inhibition must be attributable, at least in part, to subnormality in the few motoneurons which were fired by the crossed volley. An additional factor for inhibition may be subnormality in the neurons of internuncial systems which tonically barrage the motoneurons; for following the increased activity of the period of facilitation, the number of internuncial impulses impinging upon the motoneurons is reduced below the resting level.

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INFLUENCE OF SENSORY SYSTEMS ON SPONTANEOUS ACTIVITY OF CEREBRAL CORTEX¹

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THE INFLUENCE of the sensory systems of the body upon the electrical activity of cortical brain cells is manifest in a number of different ways. The simplest case is the discrete response which stimulation of a sensory nerve evokes in the cortical cells upon which the sensory tracts directly project. By utilizing such responses the areas of the cortex concerned in specific modalities of sensation have been mapped for visual and auditory, as well as for somatic sensation from the various regions of the body (3, 6).

Afferent stimulation also affects the grouping of the *spontaneous* activity of the cortical cells, as seen in the temporary interruption of the rhythmical discharges on illumination of the eyes. But even more important influences upon the spontaneous activity have been demonstrated by deafferentation of the cortex. Bishop (4) showed that in the rabbit under Dial narcosis, separation of the cortex from the thalamus abolished the 5 per sec. rhythm of the cortical cells, leaving a more rapid rhythm intact. Because of this and other similar evidence Bishop attributed the characteristic rhythmicity of the spontaneous cortical discharges to a synchronization by impulses traversing cortico-thalamo-cortical circuits. Bremer (5), found that section of the afferent pathways *below* the level of the thalamus also modified the cortical discharges. "Isolation" of the brain of the cat under ether, by transection of the brain stem, changed the fast, regular discharges characteristic of ether anesthesia to grouped periodic bursts of activity.

Under certain conditions of deep anesthesia the influence of the afferent mechanisms is also clearly evident. Adrian (1) found that stimulation of sensory nerves in the rabbit under Dial increased the frequency of spontaneous cortical discharge. We observed a similar effect in the cat under nembutal (10); and in addition, the increased activity occurred throughout the greater part of the cortex. We found also under nembutal anesthesia that separation of the cortex from the afferent pathways at levels above or below the thalamus (or destruction of the thalamus itself), completely abolished cortical activity. These experiments showed definitely that the spontaneous activity of the cortical cells depended in the circumstances on integrity of sensory pathways.

Forbes and Morison (8), recently reported experiments on the cat which led them to essentially similar conclusions. They advanced evidence that the spontaneous activity of the cortex under nembutal anesthesia had its

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origin in subcortical, possibly thalamic centers. On stimulating sensory nerves they observed a simple "primary" response in the true sensory areas, and a "secondary" response wide-spread over the cortex. Since the "secondary response" appeared identical with the spontaneous activity, both in character and in distribution over the cortex, they felt that both had a common origin in subcortical centers.

From the studies of these various investigators it seems clear that the afferent fibers to the cortex can not only initiate discrete cortical responses, but can modify and perhaps determine the pattern of grouping of the rhythmical activity which is so characteristic of cortical cells. In the experiments herein reported we have attempted to analyze the influence of the afferent systems on the spontaneous cortical activity. These studies are an extension of work reported earlier (10).

METHODS

Nembutal (sodium pentobarbital), 40-50 mgm per kg was administered subcutaneously to cats and the brain exposed widely. Leads were taken from various parts of the cortex, but oftenest well back on the posterior ectosylvian gyrus. Silver-silver chloride electrodes were connected to a suitable amplifier and the potentials recorded photographically in the usual manner by means of a Matthews oscillograph.

In some experiments ether or chloralose anesthesia was used. However, unless other anesthetics are specifically mentioned, the results described below apply to the cortex under nembutal. Sensory nerves and sensory pathways were stimulated by a device by which condenser discharges could be applied at varying strength and frequency. Special care was taken to preserve the circulation of the cortex during the various operations. This is particularly important in decerebration, a lateral approach to the brain-stem proved the best way to avoid injury of the larger blood vessels of the stem. To stimulate the thalamus, either one cerebral hemisphere was removed, or the corpus callosum was divided and electrodes put down on the surface of, or into the thalamus. The thalamic radiations were reached by the same approach.

RESULTS

When the cortex is inactive in the sense of showing little electrical activity (as it is under *deep* nembutal anesthesia), cellular discharges can be initiated by rhythmical, slow, single-shock stimulation of sensory nerves. If the sciatic nerve is stimulated at rates between 6 and 20 per min., a cortical response resembling the grouped discharges of spontaneous bursts eventually occurs after each stimulus. Once initiated, these trains of activity may continue at the frequency of the previous stimuli for several minutes after the end of stimulation. They only gradually subside and finally disappear. It is clear that under these circumstances stimulation of sensory nerves initiates the cortical activity, and is necessary moreover, to maintain continual activity.

If the cortex fires spontaneously, as occurs under lighter anesthesia, the grouped trains or bursts of activity which are characteristic of nembutal anesthesia, appear more frequently during stimulation of a sensory nerve. Stimulation of the sciatic nerve at rates between 6 and 20 per min. results in a burst usually after each stimulus (Fig. 1). With higher frequencies the bursts appear either after alternate stimuli or at shorter intervals than be-

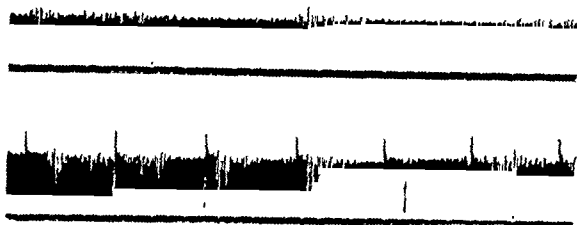


FIG. 1. Cortical response to stimulation of left sciatic nerve. Leads from right ectosylvian gyrus. Upper record control showing spontaneous activity. Lower record, stimulation at 11 per min., stimulus artefact followed by burst of activity resembling the spontaneous discharges. Cat under nembutal. Time, 1 sec.

fore, but with no definite relation to the individual stimuli (Fig. 2). In either case afferent stimulation increases the number of bursts per second and thereby the total activity of the cortex.

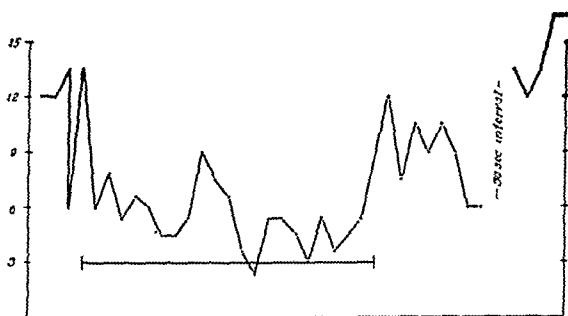


FIG. 2. Graph showing effect of stimulation of optic thalamus on cortical activity. Ordinate indicates time in seconds from onset of one grouped burst of activity (as shown in Fig. 1) to beginning of next. Each dot represents a grouped discharge. Horizontal bar marks period of stimulation of right optic thalamus at frequency of 20 per min. (once in 3 sec.). The frequency of the bursts is increased but there is not a response to each stimulus. Leads from right sigmoid gyrus. Cat; nembutal anesthesia.

After several minutes of increased activity from stimulation the cortical bursts characteristically fall back to their previous frequency, even though stimulation be continued. After the end of stimulation a long period of inactivity usually follows. These results from stimulation of a peripheral



FIG. 3. Cortical response to stimulation of the ipsilateral optic thalamus. Leads from sigmoid gyrus. Opposite hemisphere removed. Stimuli at varying frequencies; failure after 5th response. Time, 1 sec. Cat under nembutal.

nerve can be duplicated by stimulating the trigeminal nerve. Furthermore, stimulation of the central afferent systems, e.g., the optic thalamus (Fig. 2 and 3) and the thalamo-cortical pathways also induces responses which have the same characteristics.

The cortical response to slow sensory stimuli is always, under the conditions of our experiments, a burst of activity. The effect appears quite generally over the whole cortex. Furthermore, records from the two hemispheres appear similar even if the sciatic nerve is stimulated on one side only. The form of the burst is apparently quite similar in all parts of the cortex from the frontal poles back to the occipital poles and from the longitudinal fissure down to the temporal lobe.

These wide spread discharges set up by this type of slow sensory stimulation have a pattern identical with that of the spontaneous bursts. It appeared to us that the stimulation increased the rate of the spontaneous activity, rather than set up a new or different kind of response. This inference



FIG. 4. Response of cortex to stimulation of optic thalamus at high frequency between arrows (30 per sec.) resembling the discharges in human cases of epilepsy. Gradual build up of 'cusp and dart' pattern. Time, 1 sec. Cat decerebrated under ether by section of brain stem at colliculi, one hemisphere removed. Leads from ectosylvian gyrus.

is strengthened by the fact that the spontaneous bursts appear likewise identical in all the various cortical areas examined, as Forbes and Morrison pointed out. Such similarity in form and distribution of the spontaneous and induced bursts naturally raises the question whether their similarity is caused by some common mechanism.

While the cortical response to single shocks applied to sensory pathways is a burst of activity similar to the spontaneous bursts, prolonged stimulation elicits a different type of discharge. When sensory nerves are stimulated at higher frequencies (40 to 60 per sec.) for 1-5 sec., a long after-discharge follows. The pattern resembles that following prolonged direct stimulation of the cortex itself as described by Adrian (2), Dusser de Barenne and McCulloch (7), and Larrabee and Hendrix (9). Though the form of this after-discharge is unlike that of the spontaneous burst, its distribution over the cortex is the same since it is wide spread and similar in various areas. Hence, the pattern of afferent stimuli seems to determine the character of the response, but the greater part of the cortex responds, regardless of the type of stimulation.

Stimulation of the thalamus and the optic radiations at higher frequencies likewise produced similar results. There were certain exceptions. In a few animals which had been decerebrated under ether, but under conditions which we could not readily determine, a prolonged after-discharge of the kind seen in epileptic patients occurred from stimulating the optic thalamus.

(Fig. 4). We include this observation because of the implication that such a discharge may be initiated from subcortical levels.

These experiments suggest that under nembutal anesthesia the cortical discharges, both spontaneous and induced, are motivated by the activity of

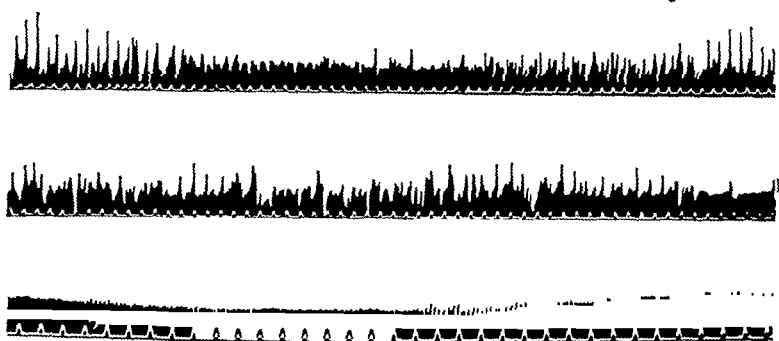


FIG. 5. Effect on spontaneous cortical activity of successive transections of upper cervical cord (middle record) and pons below 5th nerve (lower record) Upper, control. Decrease in size of spikes but preservation of grouped character. Nembutal anesthesia. Time, 0.2 sec.

sensory systems. We, therefore, studied next, the effects on the cortical activity of separating the afferent pathways at different levels of the tracts from the cortex.

Successive transections from below upward through the spinal cord, medulla and pons, causes a progressive diminution in size of the cortical discharges, but their grouped character remains unchanged (Fig. 5). On the other hand, transection of the brain-stem at the level of the colliculi, completely abolishes all cortical activity (Fig. 6). The same result follows de-

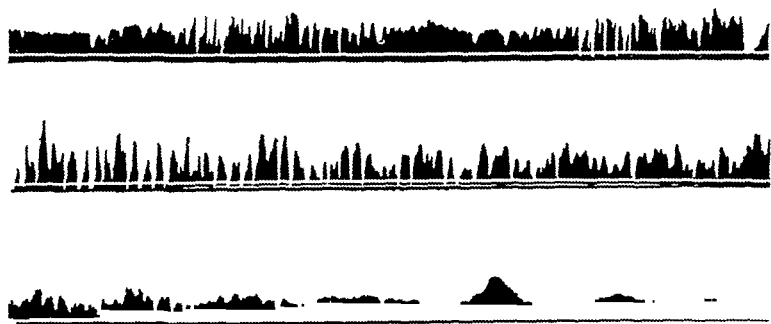


FIG. 6. Effect of transection of brain-stem between colliculi on spontaneous cortical activity of cat under nembutal. Upper record, control; middle, immediately after transection; lower, 26 sec. after section showing complete failure of cortical activity.

struction of the posterior part of the optic thalamus, or transection of the thalamo-cortical fibers in the internal capsule.

This abolition of cortical activity appears specifically due to destruction of the sensory pathways. Removal of the cerebellum, or of one cerebral

hemisphere and the frontal and occipital poles of the other, did not abolish the grouped discharge. Indeed, if all of the cortex was removed except a thin slice some 5 mm. wide, this remnant still showed typically-grouped discharges so long as it was connected to the thalamus. Furthermore, grouped discharges occurred in it on stimulation of a sensory nerve. Interruption of the thalamic connections, however, immediately stopped the discharge.

The abolition is apparently not caused by the shock of the operation of transecting sensory paths, for the more extensive operations described above have no such effect. Nor is it due to a fall in blood pressure, for the same pro-

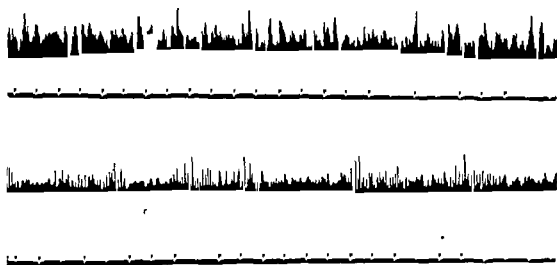


Fig. 7. The same procedure as in Fig. 6, but under ether anesthesia. Upper, control; lower, after section. The discharges become grouped but do not disappear. Time, 1 sec.

cedure under ether does not abolish the discharge. Furthermore, transection of the midbrain is not likely to cause further fall in blood pressure after the medulla has previously been cut across; yet the first mentioned procedure abolished the spontaneous discharge. Finally, the cortex was stimulated electrically, in some experiments, showing it was viable. It therefore appears to us that shock or failing circulation is not responsible for the failure of cortical discharges on transection of the afferent pathways.

The marked dependence of the activity of cortical cells on their afferent connections is due, in this case, to depression caused by the deep nembutal anesthesia. In a few experiments under lighter nembutal anesthesia the discharges reappeared after transecting the brain-stem, but they were always much reduced in size. Moreover, under other anesthetics, section of afferent pathways does not abolish the activity but only modifies it. We have confirmed Bremer's observation that under ether transection of the cat's brain-stem between the colliculi changes the fast regular rhythm to periodic grouped discharges (Fig. 7). We have found a similar modification following transection of the thalamo-cortical pathways under ether anesthesia.

DISCUSSION

These experiments show that the spontaneous activity of the cortex under deep nembutal anesthesia is dependent on the integrity of the af-

ferent systems. Destruction of the afferent pathways or of the thalamus abolishes the discharges all over the cortex. Slow stimulation of afferent pathways sets up bursts of activity which appear to us identical with the spontaneous activity both in form and in wide-spread distribution over the cortex. This marked dependence of cortical cells on afferent connections is obviously related to the special character of the anesthetic used. Under ether the cortex is considerably less dependent on the afferent fibers, for it continues to fire when these are severed. But the effect, even here, is seen in the altered character of the spontaneous activity. The refractory period of cortex and thalamus under ether and nembutal is further evidence of a differential action of these two anesthetics and may account for the character of our results. Marshall (11) found a much longer relative refractory period for nembutal than for ether.

The resemblance of the activity set up by afferent stimulation to the spontaneous bursts both in the general pattern and in the wide-spread distribution over the cortex led us to conclude that the result of afferent stimulation was an increase in the frequency of the spontaneous bursts. Forbes and Morison (8) describe these generalized bursts following sensory stimulation under nembutal anesthesia as "secondary responses," while we have considered them as increased spontaneous activity. Both we and they presume that these bursts are elicited from and shaped in the afferent systems. Forbes and Morison suggest that they originate in afferent centers, perhaps in the thalamus, rather than spreading from primary sensory cortex outward. In our experiments destruction of the thalamus stopped the cortical discharges; but obviously the thalamus cannot originate the discharges unless it receives afferent impulses from the brain-stem, for transection of the stem also abolishes the cortical discharge.

All the evidence of our own experiments and of the other workers quoted above points to the fact that the pattern of the rhythmical spontaneous cortical activity is shaped under the influence of afferent impulses to the brain. Our observations and those of Forbes and Morison emphasize the importance of this afferent control over the activity of the totality of the cortical cells. It cannot be doubted that other influences share in the synchronization of the cortical discharges; intercortical connections almost certainly play a part under normal circumstances (Adrian, 2).

Nembutal minimizes these other factors and by its specific action brings clearly to light the dominant influence of the sensory systems of the body on the generalized cortical activity.

SUMMARY

The activity of cortical brain cells in cats under deep nembutal anesthesia disappears on transection of the brain-stem at the colliculi, on destruction of the optic thalamus, or on cutting the thalamocortical radiations. Removal of the cerebellum, the opposite hemisphere, the occipital and the frontal pole does not abolish the discharge in the remaining cortex. Separa-

tion of the cortical connections to the thalamus, however, promptly stops the activity. These observations show that under nembutal anesthesia the spontaneous activity of the cortex is dependent upon the integrity of the sensory pathways.

Stimulation of sensory nerves, of the thalamus, or of thalamocortical fibers at rates between 6 and 20 per min. evokes, under nembutal, discharges which appear like the spontaneous bursts of activity, both in pattern and in widespread distribution throughout the cortex. Stimulation at faster rates (16 to 20 per sec.) for 1 to 5 sec. sets up an after-discharge lasting several minutes. This also is wide-spread throughout the cortex.

These two lines of evidence show the importance of the sensory systems of the body in shaping the character of the continuous spontaneous activity of cortical brain cells.

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CHOLINE ESTERASE IN BRAIN AND SPINAL CORD OF SHEEP EMBRYOS

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INTRODUCTION

IT HAS BEEN shown that a high concentration of choline esterase is present at synapses of the central nervous system (Nachmansohn, 1937, 1939b), as well as at the end plates of voluntary muscle (Marnay and Nachmansohn 1938). The enzymatic concentration is high enough to split a considerable amount of acetylcholine (ACh.) during the short refractory period of the effector cells. A preliminary condition is thereby fulfilled for the premise that ACh. might be involved in the transmission process of nerve impulses across central synapses and neuromuscular junctions.

If the high concentration of choline esterase at synapses has a functional significance it might be expected that a relationship exists between the time when brain centers begin to function and the time when the high concentration of enzyme appears. Such a relationship has actually been found in brains of chick embryos and newborn rats, rabbits and guinea pigs (Nachmansohn 1939b). In the brains of chick embryos, which are fairly well developed at hatching, the concentration of enzyme rises markedly during the last 4 days before hatching. In newborn rats and rabbits, which are relatively more undeveloped, the concentration is low and rises to high values during the first 3 weeks after birth, during which time the brain centers develop. In guinea pigs the concentration is high at birth, here again in agreement with the development of the brain. A similar parallelism between function and enzyme concentration has been demonstrated for neuromuscular junctions (Nachmansohn 1939a).

For several reasons it seemed of interest to study this relationship in sheep embryos. Barcroft and Barron (1939) have recently investigated the development of movements in sheep fetuses and have reviewed in connection with their own observations our knowledge of embryonic mammalian movements. Their conclusion is that movements in mammalian embryos are at first mainly local reflex responses. The next step is the domination of the more local reflexes by the reticulo-spinal system. Subsequently the development of the higher centers controlling the movements progresses, until ultimately the whole brain enters into action.

The observations on the unequal rate at which different centers of the central nervous system develop in sheep embryos suggested an investigation of the correlation between the time when the different parts begin to function and the time when the high concentration of choline esterase appears.

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That this concentration rises at an unequal rate in different centers has previously been observed (*l.c.*). However the sheep embryos appeared to be particularly suitable material for two reasons: More data on the development of movements are available in this case than for most other animals (Barcroft and Barron, 1939), and this embryo is rather large in comparison with the other embryos examined and it grows slowly, over a period of nearly 150 days. It is obvious that these conditions greatly facilitate a more detailed study than had been possible in small embryos.

METHODS

The enzymatic concentration has been determined with the manometric method of Barcroft-Warburg. The details are the same as those described previously (Nachmansohn, 1939). The symbols used are:

QCh.E. = mg. acetylcholine hydrolyzed by 100 mg. tissue (fresh weight) in 60 min.

TCh E = mg. acetylcholine hydrolyzed by the total organ (fresh weight) in 60 min.

The figures are given for acetylcholine chloride. To obtain the values for the free base, they must multiplied by 0.8.

RESULTS

A. Spinal cord. The 2nd cervical and the 8th dorsal segment have been used. The 8th dorsal segment was chosen because the corresponding roots

Table 1. Changes of concentration of choline esterase in spinal cord during growth of sheep embryos

Age in days→:	60		60		75		76		84	
	W	Q	W	Q	W	Q	W	Q	W	Q
Segment C II	10 0	11 6			17 0	9 1	30 0	9 5	42 0	10 0
D VIII	11 0	12 9	11 0	12 3	29 0	14 1	32 5	16 8	65 5	16 0
Age in days→:	89		93		97		118		136	
	W	Q	W	Q	W	Q	W	Q	W	Q
Segment C II			56 5	7 2	42 0	5 8	222 0	5 8	250 0	5 6
D VIII	78 0	12 8	60 0	13 7	65 5	8 8	260 0	9 3	202 0	5 0

W = weight in mg.

Q = QCh E.

are chiefly located in the hind limbs. The 2nd cervical ganglion was taken in order to investigate whether the rise of the enzyme concentration during development proceeds in the cephalocaudal direction. The determinations cover the period from the 60th until the 136th day, *i.e.*, near the time of birth. The whole segment without the ganglia has always been taken for the determinations.

The figures of Table 1 show that the concentration of choline esterase in

the 8th dorsal segment is high as early as the 60th day. It continues to rise, reaching a maximum between 75 and 85 days, and then decreases slowly. This decrease is obviously due to medullation of the fibers, since a large number of fibers acquire their myelin sheaths at about this time and the

Table 2. Changes of concentration of choline esterase in different parts of brain of sheep embryos during growth

Age in days:	75		76		84		89	
	W	Q	W	Q	W	Q	W	Q
cerebral cortex	70.2	1.3	23.0	1.3	30.5	2.4		
nuc. caudatus					56.0	9.2		
corp. quad. ant.	96.0	5.1	114.0	4.8	53.5	6.0		
corp. quad. post.					19.0	3.8		
retina							26.5	1.6
cerebellum	215.0	2.1	257.0	2.0			649	2.7
		T		T				T
cerebellum	215.0	4.6	257.0	5.1			649	17.7
Age in days:	97		118		136		138	
	W	Q	W	Q	W	Q	W	Q
cerebral cortex	40.0	2.4			80.0	3.4		
nuc. caudatus	91.0	12.4	98.0	22.0	78.0	32.6	102.5	35.5
corp. quad. ant.	68.5	6.5	197.0	8.3	187.0	15.5	246.0	13.5
corp. quad. post.	30.0	5.0	21.0	3.7	22.0	6.8		
retina			50.0	6.5	52.0	14.2	33.0	14.6
cerebellum			293	4.8	149	13.3	249	12.0
				T		T		T
cerebellum			3260	156	4416	587	4100	505

W = weight in mg. Weight for Q (=QCh.E.) indicates weight of tissue actually taken for experiments; weight for T (=TCh.E.) indicates weight of whole cerebellum.

myelin has a low content of enzyme. The decrease of the average concentration due to the great increase of the number of medullated fibers seems to indicate that there is no further increase of any importance in the concentration of the enzyme in the gray matter. The values of QCh.E. of 12-16 found at the maximum are indeed high if compared with the QCh.E. in the

gray matter of the spinal cord of adult dogs and ox, which are between 6 and 12.

In the 2nd cervical segment the highest value was in an embryo 60 days old. This may indicate that in this segment the maximum is actually reached earlier than in the more caudal segment. But since there are no further data available on such an early stage this cannot be conclusive at the present moment.

In one embryo which was 76 days old esterase was determined in 5 dif-

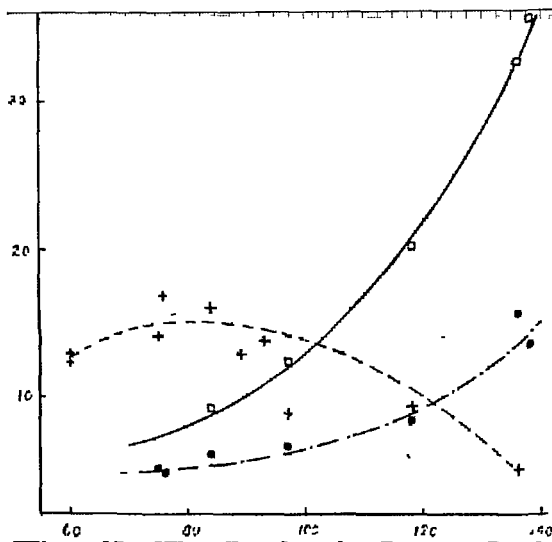


FIG. 1 Changes of choline esterase in brain (nucleus caudatus and corp quadrigem anterior) and spinal cord of sheep embryos during growth Abscissa days of gestation time Ordinate QCh E Squares, nucleus caudatus, black dots, corp quadrigem anterior, plus signs, 8th dorsal segment of the spinal cord

ferent segments. The QCh.E. values obtained were 9.5 for the 2nd cervical, 10.0 for the 5th and 6th dorsal, 12.5 for the 7th dorsal, 16.8 for the 8th dorsal and 12.0 for the 3rd lumbar segment. The weights were 30.0, 23.5, 37.5, 32.5 and 40.0 mg. respectively. It appears from these figures that the highest concentration among the segments examined exists in the 8th dorsal segment. This may be related to the fact that the number of cell bodies and synapses in this segment is higher than in the others.

B. Brain. The following parts have been examined: cerebral cortex, nucleus caudatus, corpora quadrigemina ant. and post., retina, and cerebellum. The age varied between 75 and 138 days. The figures of Table 2 show that, in contrast to the development in the spinal cord, the concentration of the enzyme is low in all these brain centers at the age of 75 days and then rises continuously and steeply. In the cerebral cortex the increase is relatively small compared with that in other parts.

It must be kept in mind that, unlike the segments of the spinal cord as used in the experiments, the parts of the brain used contain mainly gray matter. There is, therefore, no opposed effect on the QCh.E. from white

Table 3. Changes of concentration of choline esterase in some striated muscles of sheep embryos during growth

Age in days:	75		76		84		89		97	
	W	Q	W	Q	W	Q	W	Q	W	Q
leg muscle	232	1.7					326	2.6	149	2.5
intercostal muscle			71	3.9					275	4.2
extrinsic eye muscle			44	8.6	49	9.5			57	9.3
Age in days:	118		132		136		138			
	W	Q	W	Q	W	Q	W	Q		
leg muscle	664	2.1			239	1.2				
intercostal muscle	270	2.3	370	2.2			502	2.1		
extrinsic eye muscle	60	6.6	114	7.8	147	12.0				

W = weight actually used in mg.

Q = QCh.E.

matter to such an extent as in the spinal cord; but in spite of this reservation the difference between brain and spinal cord is obvious and striking (see Fig. 1).

The increase in total enzyme activity is even larger than the figures of the QCh.E. indicate, because the mass of the brain increases considerably during the same period. The total increase is demonstrated by the TCh.E. calculated for the cerebellum, as this part is the most suitable for such estimation. The TCh.E. of cerebellum rises from 5 at 75 days to over 500 before birth.

The cerebral cortex begins to develop considerably from about the 80th day on. It may be that the relatively small increase of the QCh.E. of the cerebral cortex is due to the enormous increase of the mass, whereby the increase in concentration is less apparent.

C. Muscle. The rapid development of muscle of chick embryos examined

in previous experiments covers only a few weeks. Moreover in these small muscles many nervous elements develop in a relatively small space. At the period at which end plates appear the enzyme concentration is rapidly rising. But it is difficult to ascertain in such a complex formation how far this rise is linked with the appearance of end plates.

It has been observed (Dickson 1940) that in intercostal muscles of sheep embryos motor end plates are not yet present at 100 days and have a simple form even at 137 days. These muscles and leg and extrinsic eye muscle have been examined. The figures of Table 3 show that around the period from 80-100 days the QCh.E. of leg and intercostal muscles reach the highest values. Muscle fibers have a low content of enzyme. As the relative volume of fibers becomes more important in later periods of development, the relatively high values of QCh.E. in the early period indicate that at this time a high concentration of enzyme already exists in the region of nerve endings. Recent investigations (Couteaux and Nachmansohn, 1940) suggest that a high concentration of choline esterase exists both inside and outside nerve endings at end plates. Whether the enzyme at an early stage is also localized both in and around nerve endings cannot yet be decided.

It is known that extrinsic eye muscles contain a large number of end plates. The concentration of enzyme in these muscles is particularly high at an early stage. But the number of experiments is not sufficient to permit an interpretation of the changes during growth.

DISCUSSION

The chief result of the experiments described is evidence that the high concentration of choline esterase appears in different parts of the central nervous system of sheep foetuses at different periods of embryonic development. The concentration is high in the spinal cord at an early stage (60-80th day), it is low at that time in brain centers. In these centers it rises rapidly during the last weeks before birth. This is in agreement with the development of the activity of these centers in the animals as observed by Barcroft and Barron (1939) on the development of movements. It brings new evidence of the close relationship between the time when the high concentration of enzyme appears and the time when brain centers begin to function, indicating the physiological significance of the enzyme.

Several details discussed in the text remain unexplained. *E.g.*, the work does not cover the earlier period before the 60th day, which would be of special interest for the question of a development in the cephalocaudal direction. But the experiments demonstrate the suitability of the material for such investigations.

The observation that the enzyme is highly concentrated at the region of nerve endings in muscle at an early age, long before the formation of end plates, apparently indicates that the concentration is more closely related to the function than to the histological formation.

SUMMARY

1. Choline esterase is present at a high concentration in the spinal cord of sheep foetuses at an early age of gestation (60–80th day). It is low at that time in brain centers where it rises rapidly during the last weeks before birth. The experiments emphasize the close relationship between function of brain centers and the enzyme studied.

2. In striated muscle the enzyme is also highly concentrated at the region of nerve endings at an early age long before end plates appear.

3. A remarkably high concentration is found in extrinsic eye muscles, this too at an early period.

This work was undertaken following a suggestion from Sir Joseph Barcroft and Dr. D. H. Barron, to whom I am greatly indebted for advice and help. The experiments were carried out in the Sir William Dunn Institute at Cambridge. It is a great pleasure to thank Sir Frederick Gowland Hopkins for hospitality. His stimulating interest in the work was a great encouragement.

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CENTRAL COURSE OF "RECURRENT" SENSORY DISCHARGES*

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COURSE OF "RECURRENT" SENSORY DISCHARGES

THE FIRST evidence, obtained by oscillographic methods, that impulses leave the spinal cord via the dorsal roots was presented by Matthews in 1934. In this preliminary publication Matthews identified three types of impulse discharges: (i) "sensory like," (ii) "motor like," and (iii), those conducted in small fibres.

The "sensory like" discharges were investigated further (Barron and Matthews, 1935a) in conjunction with the peculiar phenomena of intermittent conduction, already recognized by Matthews (1934). These impulse discharges, except for intermittent interruptions in the impulse series, were identical with impulse discharges recorded from sensory nerves. They entered at one fibre and after traveling some distance in the posterior columns emerged again by another to travel toward the periphery. This central pathway and its properties were studied physiologically after the entering and emergent rootlets were found for an individual sensory discharge. The physiological properties of this pathway were such that it appeared to be a single fibre, for the conduction time was that of a continuous fibre, and conduction took place in either direction at the same rate.

On the basis of these physiological observations the nature of the pathway traveled by these sensory like discharges was postulated (Fig. 2a). The impulses were assumed to travel in (a) via a normal sensory fibre (b) to ascend the cord and to leave it again via a collateral of that portion of the fibre directed toward the terminal nucleus at the end of the medulla.

As these collaterals presumably had no cell body in the ganglion of the root via which they left the cord, they ought to have remained as intact fibres in the central end of that root after the connection with the ganglion was destroyed by cutting the posterior root. Posterior rootlets were sectioned in cats in an effort to test the agreement between the physiological evidence and the structural hypothesis. Intact fibres were found in the rootlets and the anatomical evidence appeared to be in agreement with the physiological evidence (Barron and Matthews, 1935b).

This anatomical evidence has been challenged repeatedly. The careful degeneration experiments of Tower (1940) and of Hinsey (1936) and their collaborators, who find no intact fibres in the central portion of the root nor

* The histological observations were completed and the manuscript was prepared while the author enjoyed a Sterling Fellowship in the Department of Zoology, Yale University. It is a pleasure to acknowledge my indebtedness to Professor L. L. Woodruff and his staff for their hospitality and assistance.

degenerated fibres in the distal portion after section between the ganglion and the cord, cast serious doubt upon the existence of fibres of the type postulated. The question continued to recur—what is the nature of the pathway traveled by those impulses? The following experiments were undertaken during 1935 and 1936 to reinvestigate the nature of the path.

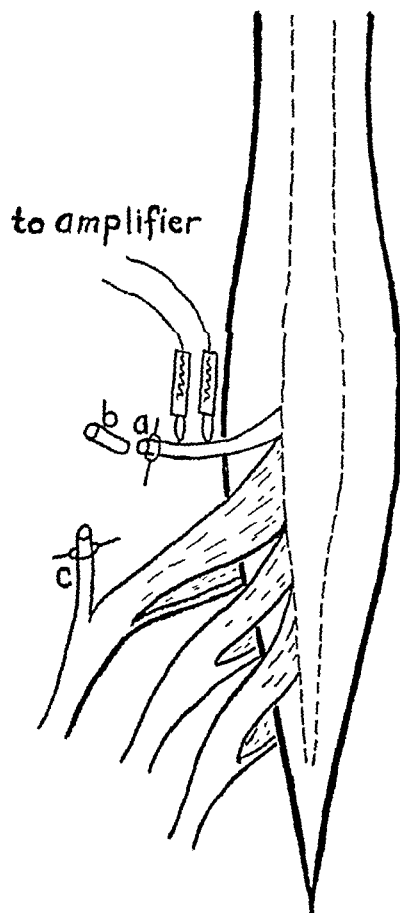


FIG. 1. A diagram illustrating the experiments designed to identify rootlets conducting recurrent discharges, and to obtain histological samples of them before and after degeneration. *a*. The central end of rootlet from which discharge was recorded before and after degeneration. *b*. The section removed at first experiment. *c*. The peripheral portion studied histologically after degeneration.

MATERIALS AND METHODS

For experimental animals rats were used instead of cats as in the original experiments. The same "recurrent" sensory discharges occurred, and the central pathway they traveled had the same properties that were described for the cat. Rats were chosen principally because they could be operated upon without danger of infection if reasonable precautions were taken. This same resistance to infection made it possible to operate upon an animal and record the action potentials in the rootlets without elaborate technical arrangements.

In practice the individual rats were anesthetized with sodium amytal. A laminectomy was performed and several tiny rootlets prepared for recording. The fibre bundles were selected from the long lumbar roots and raised free up to the entry into the cord. The distal portion was freed out as far as was conveniently possible. The rootlet was then sectioned so that a small length was taken out midway between the ganglion and the cord (Fig. 1). A colored silk thread was then tied on the tip of the central portion (*a*) and one of the same color on the peripheral portion (*c*). The sector removed (*b*) was fixed at once in 1 per cent osmic acid and labeled for identification. In a single animal usually 5 rootlets were prepared in the same way. The rat was then placed in a warm box and arrangements completed to lead from the central ends of the cut rootlets through amplifiers to a loud speaker or oscillograph. If active fibres conducting sensory-like discharges toward the periphery were found in these rootlets, a photographic record was made of the discharge and an estimate made of the number of fibres. Such a record was kept for each individual rootlet. The rootlets were then placed alongside the cord or tucked down among the roots for protection. The skin was closed over the cord with michel clips after it had been bathed in a weak solution of lysol. At intervals varying between 3 and 28 days after the operation these rats were reexamined. The cord was reexposed and the individual rootlets were lifted upon the recording electrodes. First the peripheral part was examined and then the central portion of the corresponding rootlet. Where degeneration had been allowed to proceed longer than 21 days the central portion was often so lacking in strength that it was gotten on the electrodes only with difficulty. After the observations had been made with the amplifier for action currents, the two

portions of the rootlet were removed and fixed, usually in 1 per cent osmic. In some cases the central portion was fixed in osmic acid and the peripheral portion prepared in potas-

sum dichromate for the Marchi technique. These portions of the rootlet together with those taken at the first operation were embedded in paraffin and sectioned serially at 10μ

RESULTS

Following this procedure, observations were made in over one hundred individual rootlets that were known at the first operation to have fibres conducting sensory-like discharges toward the periphery. Some of these could not be used for histological studies for the original piece removed and fixed at the operation, when studied histologically, had fibres already present in small numbers. In 87 rootlets, however, action currents were recorded in the central portions and the histological control was free from evidence of degenerating medullated fibres. In not one of the peripheral portions of these rootlets was there any evidence of degenerating fibres either after the osmic acid or the Marchi method. As a counterpart of this observation, action potentials could not be recorded from the central portion three days after it was sectioned, though it had carried recurrent sensory discharges previously. As a control against damage not directly associated with the resection, other rootlets were picked up and records made from their central ends after resection

These results are clearly incompatible with the existence of recurrent collateral fibres of the type postulated as a result of experiments on cats (Barron and Matthews, 1935). Even if they existed in small numbers the chances of detecting them ought to be extremely high, by degeneration methods in the periphery or by recording from the central end, for the majority of the rootlets contained less than 250 medullated fibres

In addition a second bit of evidence throwing doubt upon the original pathway postulated was discovered while testing the central course of the "sensory-like" discharges in rats. In a number of animals in which the centrifugal and centripetal rootlets had been identified it was possible to destroy the central pathway, not by cutting across the dorsal columns between

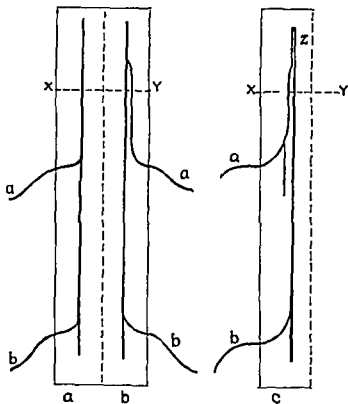


FIG 2 Diagrams of the spinal cord illustrating the possible central paths taken by recurrent sensory discharges that are interrupted by lesions ($x-y$) in the cord not placed between the entering (b) and emergent (a) rootlets (a) Section at $x-y$ would not interrupt the conduction between a and b (b) A possible modification of (a) in which lesion at $x-y$ would interrupt conduction between a and b (c) If impulse transmission took place between fibres (a) and (b) at point z lesion $x-y$ would interrupt it

the rootlets, but either above or below them (Diagram, Fig. 2). Unless the central portions of the dorsal root fibres have quite another course than that usually ascribed to them such as postulated in Fig. 2b, the result of this experiment can only mean that there are 2 fibres concerned in this central pathway and that impulse transmission is, under the conditions of the experiment, taking place between them in a one to one ratio without appreciable delay. From the earlier evidence on the cat and the rat this process might be expected to be reversible. A similar artificial synaptic relationship between the fibres of peripheral nerves in crabs has been described by Jasper and Monnier (1938). Assuming the existence of such a synapse the central pathway for the recurrent sensory discharges might be illustrated by *c* in Fig. 2. The discharge might enter via *b* and ascent to *Z* where the close approximation of the fibres would enable *b* to excite *a*. The impulses would then reach the periphery again via *a*. A cut through the posterior columns cut *x—y* would interrupt this pathway.

The phrase "under the conditions of the experiment" might be stressed for it must be remembered that the cord has been exposed and the dura opened. Under these conditions the insulation between individual fibres might be decreased or their accommodation mechanisms disturbed after the manner in which similar exposure affects the terminal portions of the posterior root fibres (Barron and Matthews, 1938). Moreover, it should be pointed out that, though these recurrent sensory discharges may be traced into and recorded from the sensory nerves to the skin of a rat after laminectomy and exposure of the cord, this has not been possible in a rat with the cord unexposed but simply anesthetized.

In conclusion, therefore, the observations presented here do not support the hypothesis that the "recurrent sensory-like" discharges are conducted through the spinal cord and out again toward the periphery in a single continuous fibre. They indicate that at least two fibres may be involved and emphasise the need for a reinvestigation of the physiological relations of their central pathway.

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INTERCORTICAL CONNECTIONS OF CORPUS CALLOSUM AS INDICATED BY EVOKED POTENTIALS

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INTRODUCTION

THE INTERCORTICAL connections of the corpus callosum have been investigated by a number of different workers using degeneration methods (Mettler, 1932, 1935; Mingazzini, 1922; van Valkenberg, 1913). The limitations of such methods are well known. Within recent years a much more convenient and precise method of studying fiber connections has been introduced, namely, the oscillographic method, in which a stimulus is applied to the afferent nerve ending and the corresponding action potential recorded from the regions which receive these fibers. The present work is an investigation of the intercortical connections of the corpus callosum by this method.

EXPERIMENTAL PROCEDURE

Twenty cats and six monkeys under barbiturate anesthesia were used in the present investigation. A rather extensive exposure of both cerebral hemispheres was made, and the dura was removed. The animals were kept in a room having a relative humidity of about 80 per cent and a temperature of about 30°C, under these conditions the cortex remained in good condition for many hours. Single electrical shocks were applied to the surface of the pia by means of bipolar silver-silver chloride electrodes about 1 mm apart, and the pick-up electrode consisted of a small chlorided silver wire lightly resting on the pial surface. The recording system is described in another paper (Curtis, 1940b), it consisted of an amplifier and cathode ray oscillograph.

RESULTS

A single electrical shock applied to the pial surface of one hemisphere will, in general, evoke a potential in the opposite hemisphere at one or more points (Curtis and Bard, 1939). A record of a typical potential is reproduced in Fig. 1. In general it is diphasic, but at times either the positive or negative phase may be so small as to make it appear to be monophasic. The height may vary from the limit of resolution (about 4 μ V) up to several millivolts. Evidence of very discrete localization may be obtained by this method. In some cases concentric electrodes, of which the outer one was only 0.25 mm. in diameter, were used for stimulation. With this arrangement it was frequently found that a point exhibiting a large response may be only 1 mm. away from a point which shows no measurable potential. Because of this fact bipolar electrodes 1 mm. apart giving a somewhat diffuse stimulation were usually used.

There can be no doubt that these responses are mediated by the corpus callosum, since they are completely abolished by section of this structure at

the mid-line. When the stimulus is repeated about once a second the callosal potential produces a standing pattern on the face of the oscillograph. Under these conditions any changes which may take place in the responses can

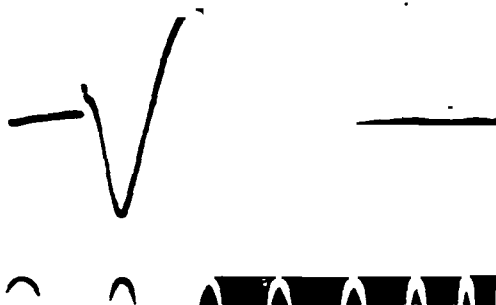


FIG. 1. Typical potential wave recorded from the surface of the pia on stimulation of the symmetrical point of the opposite hemisphere. Middle suprasylvian gyrus of cat. An upward deflection indicates a surface negative potential. Time marks 60 cycles.

easily be observed. If the cerebral hemispheres are separated slightly at the mid-line, a stimulating electrode can be placed directly on the callosum. Since stimulation here gives exactly the same type of potential as is observed when the appropriate point in the opposite hemisphere is stimulated, it is a procedure which may be employed to determine the path of the fibers in the callosum. When the region in the callosum containing the fibers connecting any two points has thus been determined, a small cut there immediately abolishes

the electrical response to stimulation of the related contralateral point, but may not affect potentials at neighboring points.

The largest and most readily detected potentials are those obtained when stimulating and recording electrodes are placed on symmetrically situated points of the two cortices. Thus it is possible to determine the relative abundance of callosal connections between symmetrical points in different

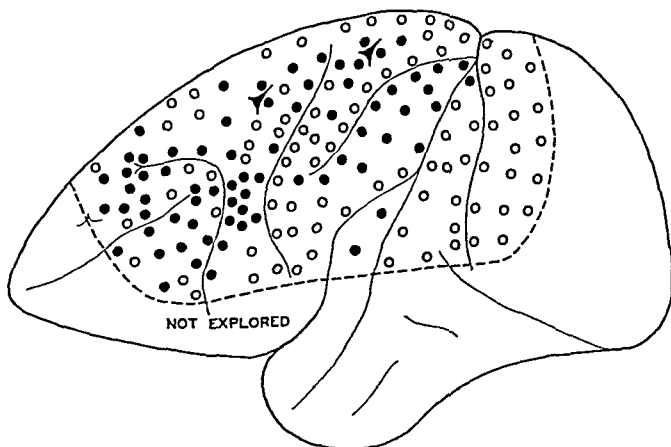


FIG. 2. Lateral view of left cerebral cortex of a monkey. Marks indicate the relative magnitudes of potentials evoked at different points by stimulation of the symmetrical points of the opposite cortex. Results of an experiment on one animal. ● Large potentials; ○ medium potentials; ○ small or no potentials.

regions by systematically exploring the cortex with a constant stimulus, always keeping stimulating and pick-up electrodes on symmetrically situated points and maintaining constant amplification. The height of the potential may then be taken as a rough measure of the extent of callosal association between the two points. The results of such an experiment on a monkey are shown in Fig. 2, where solid circles indicate points which gave large potentials, dotted circles medium potentials, and open circles weak or no potentials. Figure 3 is a composite map drawn from results obtained in about 15 experiments on cats. It

will be seen from these figures that the distribution of callosal fibers between different cytoarchitectonic areas, or even between different parts of the same area is by no means uniform. Whereas the map of Fig. 2 shows the results obtained in the case of only one animal, each of the experiments on monkeys showed the same general pattern of distribution. The absolute magnitude of the potentials recorded from different monkeys varied considerably, but the relative heights of these potentials

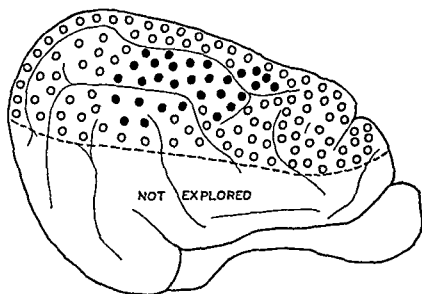


FIG 3. Lateral view of right cerebral cortex of a cat. Marks indicate the relative magnitudes of potentials evoked at different points by stimulation of the symmetrical points of the opposite cortex. Summary of results obtained in fifteen experiments. ● Large potentials; ○ medium potentials; ○ small or no potentials.

at different points were fairly uniform. The same is true of the cat, but the absolute magnitude of the potentials in the cat is much less than in the monkey, and in any individual cat there may be a considerable fraction of the exposed cortex from which it is impossible to obtain measurable potentials. However, by the use of a number of cats it has been possible to show that there is no part of the cortex which was explored which does not give these potentials at symmetrical points. In the case of the monkeys, the same is true with the exception of the striate cortex, area 17 of Brodmann; it has not been possible to obtain any indication of a potential from any part of this area on stimulating a symmetrical point.

It has been found (Curtis, 1940a) that the application of a convulsant drug such as strychnine or picrotoxin to the surface of the pia under the recording electrode will in general enormously enhance the recorded potential. This fact can often be used as a means of amplifying a potential which was previously difficult or impossible to record with certainty, but sometimes the enhancement occurs only when the potential is initially quite large. The strychnine effect has been used to check some of the results described above. It is apparent that the absence of potentials from area 17 of

the monkey does not necessarily indicate that there are no connections, but it does give presumptive evidence that if present, they must be rather weak.

In addition to the potentials observed when stimulating and pick-up electrodes are situated at symmetrical points, potentials are often observed in quite different regions. This effect is much more pronounced in the monkey than in the cat. Thus in order to determine all the intercortical connections of the callosum it would be necessary to stimulate systematically each point of one cerebral cortex and for each position of the stimulating electrodes to explore systematically the entire opposite hemisphere with the pick-up electrodes. This has been done for only a relatively few selected points, and the results of one such experiment are shown in Fig. 4. Here the

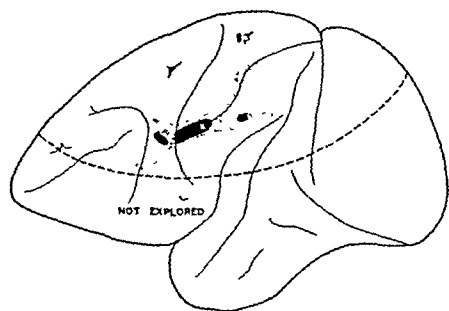


FIG. 4. Lateral view of left cerebral cortex of a monkey in which are indicated the relative magnitudes of potentials obtained as a result of stimulating the point on the right cortex symmetrical to the one marked X. The degree of shading indicates the relative magnitudes of the potentials obtained in this experiment.

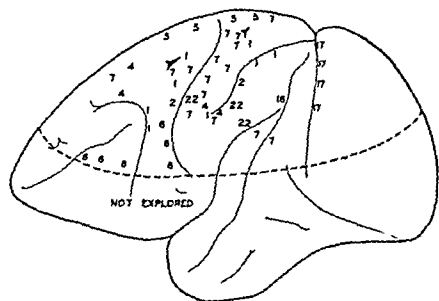


FIG. 5. Lateral view of left cerebral cortex of a monkey. The numbers indicate areas of Brodmann which send callosal fibers to the region containing the number.

stimulating electrodes were on the hemisphere opposite the one shown and at a point symmetrical to the one marked X. The degree of shading indicates the relative heights of the potentials. It is obvious that the problem of representing such data from a number of experiments is a difficult one. However, this has been attempted in the map of Fig. 5, which only indicates projections to regions other than the cytoarchitectonic area within which the stimulus was applied. The numbers refer to areas of Brodmann (1909) which send callosal impulses to the region where the number is situated. It should be pointed out that this map by no means implies that all points in an area project to the points indicated, but when any region, however small, was found to project to another cytoarchitectonic area, it was indicated. These regions were sometimes only one or two square mm. in size. Since by far the most numerous connections are to corresponding cytoarchitectonic areas, the map of Fig. 5 is a gross over-simplification of an exceedingly complex system of interconnections.

The question immediately arises whether these inter-areal connections

are by means of direct fibers in the callosum or depend on intracortical spread of activity. All evidence at the present time points to the former alternative. In the first place, no difference can be detected in the latency of the response at various places, and in the region between two points giving relatively large potentials there may be no measurable response. Furthermore when stimulation on one side evokes two large potentials, one at the contralateral symmetrical point, *A*, and another at a point, *B*, some distance from *A*, the isolation of *B* by cutting through the cortex around it, does not affect either potential. In a general way fibers joining any two points take the shortest path in the callosum between these points. In one experiment on a monkey a point in the anterior part of area 6 gave a good potential in response to stimulation of a point in the contralateral area 7. Two fairly large sagittal cuts were then made in the callosum at the midline, one medial to area 7 and one medial to area 6, leaving only a small bridge of intact callosal tissue on a line between stimulating and pick-up electrodes. The recorded potential was unaffected by this procedure, but it was completely abolished when the remaining part of the callosum was cut. This evidence points rather strongly to the existence of direct callosal connections between all points indicated in Fig. 5. When two or more points which are quite close together exhibit large potentials in response to stimulation of a contralateral point, it is difficult to determine whether the multiple response is due to separate callosal fibers to each point, or to intracortical spread from one point to another. Employment of the same procedure as outlined above indicates that, as in the case of widely separated points, there are separate fiber connections.

DISCUSSION

The evidence presented here is in good agreement with previous work. However, the lesions which have been made in attempts to study this problem by following degeneration were so large relative to the area stimulated in these experiments, and the consequent degeneration so extensive, that comparisons are difficult. Mettler (1932, 1935) found the greatest degeneration in regions symmetrical to the lesions, and this led him to a generalization which is abundantly confirmed in these experiments. The only significant point of difference between the results of the present work and those of Mettler involves the question of callosal connections between the striate areas of the monkey. Mettler (1935a) reported the occurrence of degeneration of callosal projections from area 17 of one cortex to the corresponding region of the other hemisphere, but the results of the present study are entirely negative in this respect. Other investigators have denied the presence of callosal connections between these areas (van Valkenberg, 1913; Mingazzini, 1922). This point is of interest in reference to theories of binocular vision. It was pointed out above, and should be emphasized here, that the absence of potentials does not necessarily mean that there are no connections. It would, however, seem fairly safe to conclude that if such callosal

connections exist, they must differ markedly from those which relate other symmetrical areas. There are abundant connections between the visual association areas, and also between the visual motor areas of the two hemispheres. It is interesting to note (Fig. 3) that the experiments on cats revealed the existence of fairly strong callosal connections between the two striate areas. This is in agreement with the results of Poliak's (1927) anatomical studies. It is possible that in the cat the visual projection and visual association areas are not as differentiated anatomically as they are in the monkey.

The maps showing the relative strengths of callosal potentials at symmetrical points (Fig. 2 and 3) are interesting in that there seems to be no consistent correlation between the function of an area and the strength of the symmetrical callosal connections. Again it should be emphasized that the size of the potential may or may not be a true index of the strength of the callosal connections. The position of the majority of the endings in the cortex would undoubtedly affect the size of the potential as much as the density of the callosal fibers. Micro-electrode studies in the cat (Curtis, 1940b) seem to indicate that the positions of the endings of callosal afferents within the cortex are approximately the same in all areas investigated, but relatively few areas have been explored in this way in the cat, and none in the monkey.

The map of Fig. 5 shows that there seems to be no particular relation between the function of an area and its callosal projections to other areas. For example it might be expected that there would be abundant callosal connections between area 4 and area 6, but such is not the case. Of the cortical regions explored, area 7 appears to have the most abundant callosal projections to other areas.

The question arises of course as to how much of the observed potential is due to antidromic stimulation. Stimulation of a given region of one hemisphere may evoke a potential in a totally different region of the opposite hemisphere but if stimulating and pick-up electrodes are reversed, no potential can be observed. For this and other reasons it has been concluded (Curtis, 1940b) that antidromic impulses play a very minor role in the production of the potential as recorded at the surface of the pia.

It is interesting to speculate as to what these results may indicate concerning the function of the corpus callosum. The first indication is that the chief connections between the hemispheres are to symmetrical points and that other connections are probably of relatively minor importance. A clew to its function may lie in the fact that areas of the cortex which control the motor movements of muscles which usually function synchronously on the two sides of the body have abundant callosal connections. For example, the motor face, head, and trunk areas appear to have stronger connections in general than the arm, leg, or hand areas.

SUMMARY

In monkeys and cats under barbiturate anesthesia single electrical shocks were applied to the cortex of one hemisphere and the other cortex explored for evoked potentials. The only regions not studied were those which cannot readily be exposed by removal of the calvarium. In general it may be said that localized stimulation of one cortex gives rise to distinct evoked potentials in one or more specific places on the other cortex. The magnitude of the response is quite variable; it may be as large as several millivolts. In general the waves are diphasic and sometimes have a duration as long as 100 msec.

The largest and most readily detected potentials are those obtained when the stimulating and recording electrodes are placed on symmetrically situated points of the two cortices. In the cats studied all regions yielded potentials from symmetrical contralateral stimulation. With the exception of the operculum of the occipital lobe (area 17) the same was true of the monkeys. Localized stimulation within certain areas may evoke potentials over a considerable portion of the corresponding contralateral area and sometimes in other contralateral areas. The responses obtained under the conditions of these experiments are mediated by direct callosal fibers and are not dependent upon intracortical spread of activity. In general the evoked potentials are quite sharply localized. They are fairly reproducible from animal to animal.

These effects are completely abolished by section of the corpus callosum.

The author wishes to express his appreciation to Dr Philip Bard for his advice in all parts of this work

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AN ANALYSIS OF CORTICAL POTENTIALS MEDIATED BY THE CORPUS CALLOSUM

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INTRODUCTION

IN ATTEMPTING to analyze the functions of the cerebral cortex the recording of electrical potentials generated by the cortex has proved to be very valuable. For the most part such studies have been carried out by placing electrodes either on the skull or on the exposed surface of the pia and recording the spontaneous activity. Analyses of these potential changes have proved to be difficult for the reason that any or all of the elements of the entire central nervous system may be contributing directly or indirectly to the activity. In many respects a much more satisfactory method is that of studying the potential changes of the cortex which result from stimulating afferent fibres or some sense organ. Such studies have been carried out by a number of investigators on various cortical afferent systems. Perhaps the simplest anatomical picture of a corticopetal system is presented by the inter-cortical fibers of the corpus callosum.

The cell bodies of the axons which form the callosum lie in layers III, V, and VI of the cortex (Pines and Maiman, 1939). On entering the cortex of the opposite hemisphere the callosal fibers ramify profusely in layers IV to II and end in layer I (Kölliker, 1896; Lorente de Nó, 1922, 1938). If one cerebral hemisphere is stimulated with single electrical shocks (Curtis and Bard, 1939; Curtis, 1940b) potentials will be evoked at one or more points on the surface of the opposite hemisphere, and these potentials are mediated by the corpus callosum.

Whenever a cortical potential is obtained as a result of afferent stimulation, one has direct evidence that there is a neural connection between the nerve being stimulated and that point on the cortex. However, this gives very little evidence concerning the mechanism whereby this potential is produced. A number of investigators (Bishop and O'Leary, 1936; Lorente de Nó, 1939; Renshaw, Forbes and Morison, 1940), by inserting micro-electrodes into various parts of the central nervous system, have recorded potentials of discrete local origin. An analysis of such data has furnished much valuable information concerning the origin and function of various neuron systems. The present work is an attempt to make such an analysis of the callosal system.

PROCEDURE

Twenty-two cats, under barbiturate anesthesia, were used in these experiments. The calvarium and dura were removed over both cerebral hemispheres. The head of the animal was rigidly fixed in a holder to which various electrodes were attached in such a way that

any one of them could be placed on any part of the exposed cortex and one of them inserted to any desired depth. Single electrical shocks were applied to the pia surface through an isolation transformer by means of silver silver chloride bipolar electrodes about 1 mm apart.

The micro electrodes which were used for recording consisted of glass micropipets, drawn to an inside diameter of about 50μ and filled with a gel of agar in Ringer's solution, into which a chlorided silver wire dipped. Some of the electrodes were bipolar, consisting of two such capillaries fused together, one of which was broken off about 200μ shorter than the other. The advantages, applications, and limitations of such electrodes have recently been discussed thoroughly by Renshaw, Forbes and Morison (1940) and will not be repeated here. Suffice it to say that with these electrodes it is possible to obtain responses of very local origin and that both monopolar and bipolar recording should ultimately lead to the same conclusions. All conclusions reached in this work were based on examinations of records taken by both types of electrodes.

The electrode holder for the micropipets was so arranged that they could be inserted at any angle and the depth of insertion accurately measured by means of a scale attached to the slider of the holder. The position of the electrode in the brain was also determined by subsequent histological examination of serial sections. A reference point for the depth measurements was established at the end of each insertion by placing beside the pipet a fine glass rod which was made lightly adherent to it by means of a small amount of mineral oil. On inserting the pipet the rod followed only as far as the surface of the pia. On removing the pipet the depth of insertion of the needle for that particular setting of the scale of the electrode holder was given by the distance between the end of the rod and the end of the needle. From measurements made on serial sections of the brain it was thus possible to locate in terms of each scale setting the position of the electrode in relation to the various cortical layers and the underlying white. It was determined that these measurements were accurate to about 100μ . It should be emphasized that the electrodes were very small and had very sharp points. On piercing the pia they caused only a slight momentary indentation. Thereafter the electrode could be raised or lowered without causing an appreciable movement of the cortex. For these reasons the depth measurements made by the method indicated above proved to be more reliable and convenient than any of several other methods which were tried in attempts to mark the position of the electrode in the cortex for subsequent histological identification. The electrodes were so small that inserting them caused no measurable change in the callosal potential recorded separately at the surface. The electrode could be raised or lowered as often as desired without changing the potential as recorded from the micropipet at a particular scale setting even when a change of 100μ caused a radical change in the potential picture.

For recording the potentials a direct coupled amplifier with common mode degeneration was used in conjunction with a cathode ray oscillograph. A condenser was usually inserted between the second and third stages of the amplifier to eliminate drifts. Whenever there was any doubt about the true wave form this condenser was switched out and the potential recorded with the direct coupled amplifier. The micro electrodes had a resistance of several megohms. Since this resistance in conjunction with the capacity of the leads from the amplifier to the preparation is an arrangement which would shunt out any very rapid potential changes occurring in the neighborhood of the electrodes this source of error was eliminated by mounting an impedance changer on the carriage of the electrode holder next to the micropipet.

Changes in the size and shape of the callosal potentials recorded from the surface of the pia occurred from time to time. Therefore a separate electrode was placed on the surface of the pia over the region being explored in depth with the micropipet. It was connected to a separate amplifier and oscillograph. If the surface potential changed during a series of observations made with the micro electrodes the series was either repeated or discarded.

RESULTS

A normal callosal potential, as recorded from macro electrodes on the surface of the pia, is shown in the uppermost record of Fig. 1. There is, of course, considerable variation in the size and shape of such potentials. In general, they consist of an initial surface positive component followed by a surface

negative component. The entire wave may last as long as 100 msec. The two components are separate and distinct. That such is the case is shown by (i) their different sites of origin as revealed by micro-electrodes and (ii) the differential action of drugs. If a small quantity of a narcotic such as nem-

butal or cocaine is placed on the pia over a small area receiving callosal impulses, the negative component of the potential is obliterated, but the positive remains unchanged (Curtis, 1940a). On the other hand, if a small amount of a convulsant drug such as strychnine, picrotoxin, metrazol, etc. is added in the same way, and enormous enhancement of the negative component occurs (Bartley, O'Leary and Bishop, 1937). These phenomena are shown by the records of Fig. 1, all of which were taken from the same point on the pia. The first is the normal, the second was obtained after the local application of nembutal, and the third after application of strychnine. Before the record showing the effect of the strychnine was taken, the effect of the nembutal had completely worn off and the wave shape had returned to normal. Thus it is possible, by means of drugs to separate the two components and to analyze each separately.

No fast axon-like spike potentials such as those recorded by Renshaw, Forbes and Morison (1940) from micro-electrodes in the hippocampus were ever obtained from either the cortex or the callosal fibers leading to the cortex. Failure to observe such potentials during

numerous experiments means either that the observer was extremely unlucky or that the elements contributing to the potentials are numerous and small in comparison to the size of the electrodes.

Considering first the initial positive wave, Fig. 2 shows records taken with a micropipet at various depths below the surface of the pia after the local application of nembutal. Subsequent histological examination showed

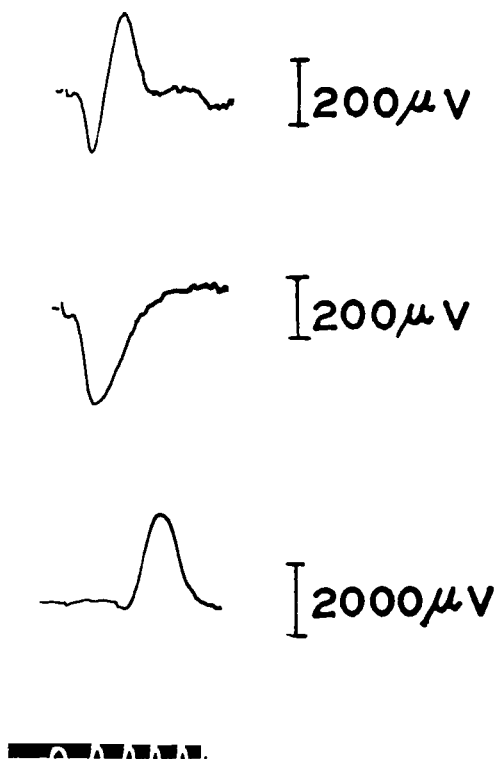


FIG. 1. Potentials recorded with a macro-electrode on the surface of the middle supra-sylvian gyrus in response to contralateral cortical stimulation. The upper record is a typical normal wave; the center record was taken at the same point after the local application of 6.5 per cent nembutal at the recording electrode; and the lower record after application of 1.0 per cent strychnine sulfate. Upward deflection indicates a surface negative potential. Time marks, 60 cycles.

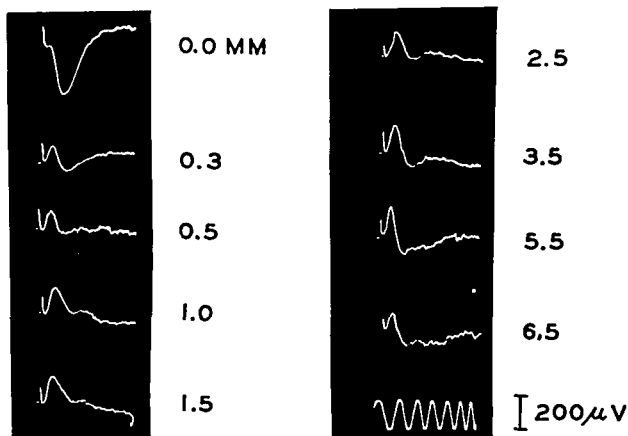


FIG 2 Potentials recorded with a unipolar micro electrode from various depths below the surface of the middle suprasylvian gyrus in response to contralateral cortical stimulation. All records were taken after the local application of 6.5 per cent nembutal at the site of entry of the recording electrode. An upward deflection indicates that the active electrode was negative with respect to the indifferent electrode. Time record, 60 cycles.

that the electrode had gone straight down into the white matter of the gyrus as in the section shown in Fig 3. At this point the cortex is 2.0 mm thick. When electrodes were inserted in the gray matter of the gyrus just medial and lateral to the needle tract from which these records were taken, no activity was recorded; it is therefore clear that they were not simply due to the presence of large potentials originating at a distance. It will be noticed from the records of Fig 2 that when the electrode is completely in the white matter, the wave is predominantly negative. As the electrode leaves the white matter and enters the gray there is no discontinuity in the records until a point about 0.5 mm

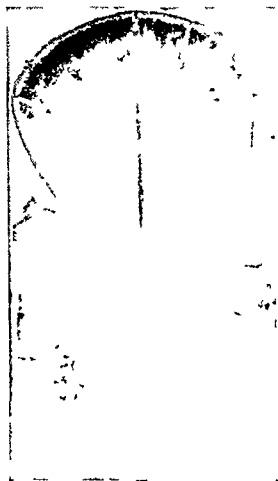


FIG 3 Frontal section of middle suprasylvian gyrus showing a micro electrode track.

from the surface is reached. Here the wave starts to reverse, but this reversal is not complete until the electrode is only about 0.1 mm. below the surface, *i.e.*, in the upper part of layer I.

These records show that no particular cell layer is responsible for the production of the initial positive wave; one would expect a marked change in the record as the electrode went through this layer, if such existed. On the other hand, it is possible to interpret these records as being due to summated axon spikes. This problem has been treated theoretically by Wilson, Macleod and Barker (1933) and the applications of their theory to phenomena in the central nervous system have recently been discussed by Lorente de Nó (1939). They showed that in a volume conductor (*e.g.*, Ringer's fluid), records taken between an indifferent electrode and one in the center of a fiber tract consisting of similar fibers discharging synchronously will show a triphasic wave. The first phase is positive and small, the second negative and large, and the third positive and small. It may be pointed out that if the fibers are firing somewhat asynchronously, the first and last phases, being small, would be obscured, leaving an almost pure negative wave.

At the present time there is no adequate theoretical treatment which can be used to interpret the significance of potential changes recorded from an electrode in the neighborhood of the end of a fiber tract immersed in a conducting medium. In order to investigate this case and to verify the above theoretical predictions a model of the callosal system was set up which consisted of a frog nerve suspended in a beaker of Ringer's fluid with both ends just reaching the surface a few centimeters apart. One end of the nerve was stimulated and records were taken by means of a micropipet inserted in the nerve at various points along its axis. When the electrode was in the nerve some distance from the end a triphasic wave was recorded in which the negative component was by far the most prominent. This is in agreement with the theoretical predictions. When the electrode was on the surface at the end of the nerve, a pure positive wave was obtained. Further, when this end of the nerve was lowered in the solution and the electrode placed at its end, a pure positive wave was again recorded. This shows that if a nerve impulse is traveling toward an electrode, but does not quite reach it, a positive wave will be recorded. It will be seen that the records of Fig. 2 fit this nerve model picture almost perfectly. Here on raising the electrode the negative wave of the callosal fibers does not change until a depth of about 0.5 mm. is reached, where a positive component starts to enter, indicating that some fibers have ended before they get to this point, but the fact that it does not completely reverse until the upper part of the first layer is reached shows that a considerable fraction of the fibers extend this far.

Turning to the origin of the negative part of the cortical wave as amplified by strychnine, Fig. 4 shows records taken with a monopolar micropipet at different depths in a strychninized area. Here it will be seen that reversal takes place at a depth of about 0.8 mm. and that the wave remains

positive as far as it can be followed in the white matter. The depth at which the reversal occurs seems to be quite variable.

The theoretical interpretation of the records taken with strychnine is not completely clear, but several points seem quite definite. The wave is always negative to a surface electrode and positive to an electrode in the deeper cortical layers and adjacent white matter, and the transition from one to the other is not a smooth process. If the electrode is placed at the point of minimal potential and the amplification increased, a number of small asynchronous waves can be seen, some positive and some negative. This indicates that a large number of cortical elements contribute to the production of the potential as measured at the surface, and these elements may be some distance apart in the cortex. It is possible to explain this potential on the basis of summated axon action potentials, just as in the case of the initial positive potential. It seems to indicate impulses which are initiated in the first cortical layer and travel to the deeper layers, as far as layer V. The positive potential in the deeper layers would then be due, as before, to impulses traveling toward the electrode but stopping before they reach it.

On applying strychnine to the cortex while recording from a surface electrode, there is, in addition to the enormous enhancement of the negative wave, some enhancement of the initial positive wave. If, after an area has been strychninized for some time, a small amount of cocaine is added, the negative wave disappears within a few seconds, leaving the enhanced positive wave. As the cocaine penetrates deeper into the cortex the enhancement of the positive wave gradually

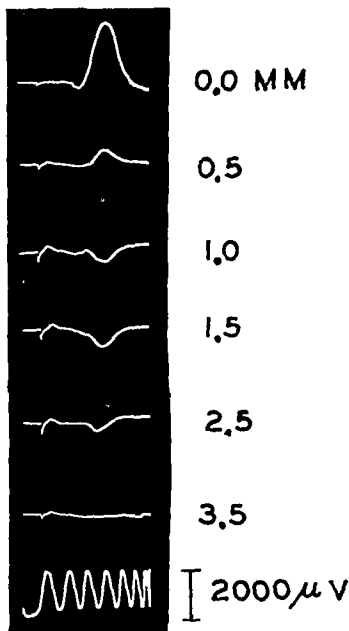


FIG. 4 Potentials recorded with a unipolar micro-electrode at various depths below the surface of the middle suprasylvian gyrus in response to contralateral cortical stimulation. All records were taken after the local application of 10 per cent strychnine sulfate at the site of entry of the recording electrode. An upward deflection indicates that the active electrode was negative with respect to the indifferent electrode. Time record, 60 cycles

disappears. After about 3 min. the initial positive wave reaches normal size. It is difficult to follow the enhancement of this wave within the cortex, but it is believed that it reverses in sign at a level about 0.1 mm. below the surface and is measurable to a depth of about 0.6 mm. Thus this enhancement can be explained as being due to the discharge of an additional group of fibers originating in layers II and III of the cortex and terminating in layer I.

Each of the convulsant drugs which was used has its own peculiarities. While no systematic attempt has been made to differentiate between them, a few of the more striking dissimilarities may be noted. *Picrotoxin* is the most potent, is slow to act, and its effect lasts for several hours. *Metrazol* acts rapidly, but exerts its effect for only a few minutes. *Strychnine*, *acetylsalicylic acid* (aspirin) and *camphor monobromide*, listed in order of potency, act in 5-15 minutes and produce effects for 30-40 minutes.

DISCUSSION

The evidence presented seems to point strongly to the view that convulsant drugs lower the threshold for synaptic transmission, and that narcotic drugs block synaptic transmission before they block conduction in axons. These ideas, of course, are by no means new, and the drugs were used here only to facilitate the interpretation of the normal records.

The conclusion that the afferent fibers of the callosum ramify in the second and third cortical layers and end in the first layer is in good agreement with the anatomical findings of Kölliker (1896) and Lorente de Nó (1922, 1938), but is in disagreement with those of Kappers, Huber and Crosby (1936) who state that the fibers of the corpus callosum end in layer III.

Undoubtedly cortical stimulation sets up antidromic conduction in many callosal fibers. This phenomenon can hardly affect the conclusions drawn concerning the origin of the second or postsynaptic part of the wave. The cell bodies of callosal fibers lie in layers III, V and VI (Pines and Maiman, 1939), and probably have dendrites and recurrent collaterals reaching as far as layer II (Lorente de Nó, 1938). It is therefore possible that the initial (surface positive) potential may be due in part to antidromic impulses. However, in studies on the monkey (Curtis, 1940b) stimulation of a point, *A*, on one side, sometimes produced two large potentials on the contralateral hemisphere, one at the symmetrical point, *B*, and another at a point, *C*, some distance from *B*. When the stimulating electrodes were placed at *C* and the recording electrode at *A*, no potential change was recorded. For this reason it has been concluded that antidromic conduction plays at most only a minor rôle in the production of the potential as recorded at the surface. The conclusion that callosal afferents extend to layer I is thus not invalidated by the possibility that cortical stimulation may set up antidromic impulses in callosal fibers. On the other hand, the conclusion that callosal afferents ramify in layers II and III is not conclusively proven by the present work.

The relatively long delay which sometimes occurs between the arrival of the afferent impulse and the initiation of the evoked strychnine spike is interesting. When a convulsant drug is placed on the cortex, the first effect is a gradual increase in the size of the negative wave as recorded from the surface. When this wave reaches a size several times its initial value an enhancement of the positive wave can usually be detected, and since the latency to crest is also increased the change must be due to impulses arriving somewhat later than those which cause the original positive wave. After some time (10 to 15 min. for strychnine) two positive waves, about 20 msec. apart, are seen in the records; sometimes the interval is as great as 100 msec. The first of these is the original positive wave; the second is the initial portion of the strychnine spike. When these two waves are separated it can be shown, by varying the strength of the stimulus, that the strychnine response is all-or-none; such, however, is not the case in the early stages. Investigation of the initial positive part of the strychnine response by means of micro-electrodes suggests that this potential is due to a discharge of impulses to the surface from about the level of the third layer. On this basis a typical strychnine spike will be produced only after sufficient time has elapsed to allow strychnine to penetrate to this level. The results obtained with strychnine in this work are in agreement with those of Dusser de Barenne and McCulloch (1938). When picrotoxin was placed on a point in the motor cortex, a typical "strychnine" response to contralateral cortical stimulation was produced in about 20 min., but 40 to 60 min. elapsed before a muscle twitch ipsilateral to the stimulated side was observed as a result of this response, and no difference was recorded in the potential wave when the twitch occurred. That the muscle response observed was due to activation of the contralateral motor area was demonstrated by its disappearance after local applications of nembutal to this area. Presumably it took 40 to 60 min. for the picrotoxin to penetrate to the deeper cortical layers where it facilitated conduction across synapses involving cortical efferent neurons.

Taken together the results presented in this paper indicate that the typical response to the application of a convulsant drug to the cortex originates in the third layer, is conducted to the first layer by interneurons where synaptic connection is made with other interneurons which conduct it to the deeper cortical layers where in turn synaptic connection may be made with cortical efferents.

SUMMARY

In cats under barbiturate anesthesia single electrical shocks applied to the cortex of one cerebral hemisphere evoke potentials at one or more points on the cortex of the other hemisphere. These responses are mediated by the corpus callosum. The potential wave is typically diphasic; it is composed of an initial surface positive component lasting about 15 msec. and a surface negative component lasting about 75 msec. If a convulsant drug such as picrotoxin is applied to the surface of the pia under the pick-up electrode the

negative component is greatly increased in magnitude and the positive component is increased slightly. If an anesthetic drug such as nembutal is applied, the negative component is completely obliterated but the positive component undergoes no change.

By inserting micro-electrodes to various depths in the cortex and underlying white matter during the action of convulsant and narcotic drugs it has been possible to gain some knowledge of the origin and course of the impulses which give rise to the potential changes recorded from the pial surface. The results indicate that the ascending fibers of the callosum ramify in the upper layers of the cortex and end in the first layer where they make synaptic connections with descending interneurons which lead to the deeper cortical layers. The ascending fibers give rise to the surface positive component of the wave, the descending internuncial fibers to the surface negative component.

The author wishes to express his appreciation to Dr. Philip Bard for his advice in all parts of this work.

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FUNCTION OF MESENCEPHALIC ROOT OF FIFTH CRANIAL NERVE*

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THE MORPHOLOGY of the cells giving rise to the mesencephalic root of the Vth cranial nerve has been carefully studied by many observers. The resemblance of these cells to the unipolar primary sensory cells of the cerebro-spinal ganglia was noted by Allen (1919), Clark (1926), Weinberg (1928), Schneider (1928) and Sheinin (1930). Clark (1926) demonstrated the close similarity of the large cells of the mesencephalic nucleus and those of the spinal ganglia which Warrington and Griffith (1904) demonstrated to be connected with muscle spindles. Because of this resemblance, and because of the peripheral distribution of the majority of the mesencephalic root fibers to the masticator branches of V, the consensus of opinion of workers on this subject has been that the mesencephalic root represented primary sensory fibers mediating muscle sensibility from the muscles of mastication (Johnston, 1909; May and Horsley, 1910; Willems, 1911; Kosaka, 1912; Allen, 1919, and Thelander, 1924). Furthermore, it was recently demonstrated that mesencephalic root fibers, in addition to passing peripherally with the masticator branches of V, pass into those purely sensory branches (superior alveolar, inferior alveolar and palatine nerves) which supply deep sensation to the teeth, gums and hard palate (Corbin, 1940). Central collaterals from the mesencephalic root were seen passing to the motor nucleus of V by Cajal (1896), Wallenberg (1904), May and Horsley (1910) and others.

Pfaffmann (1939) obtained action potentials from the superior alveolar nerves after touch or pressure applied to the teeth, gums and adjacent lips. The impulses were slow to adapt and conducted quite rapidly, which would indicate that they were mediated by fairly large myelinated fibers. Removal of the dental pulp and destruction of the nerves in the apical canal by cautery did not affect these responses. Pfaffmann concluded that the majority of the endings giving rise to these impulses are located in the periodontal membrane. Lewinsky and Stewart (1937) found that the larger myelinated fibers formed peculiar spindle-like endings in the periodontal membrane, and concluded, as a result of the work of Stewart (1927), that these spindles reacted primarily to pressure stimuli.

Pfaffmann also obtained strong and sudden movements of the mandible on sectioning the maxillary nerve. In addition to this observation, Sherrington (1917) found that blunt pressure stimulation of the gums bordering the teeth of both the upper and lower jaws, of the tooth crown, as well as of the hard palate, caused reflex opening of the tonically closed jaw in the decerebrate cat, involving reflex inhibition of the jaw-closing muscles as well as stimulation of the opener muscles. The evidence presented by these two workers, in addition to his own histological findings, led Corbin (1940) to postulate mediation of the afferent limb of this reflex arc by the mesencephalic root fibers which he found in the sensory branches of V. Certain workers have assigned other functions to the mesencephalic root cells. Freeman (1925), Sheinin (1933) and Woollard (1931) suggest, on the basis of studies on chromatolytic mesencephalic nucleus cells, that this nucleus is the source of the sensory fibers to the extrinsic ocular muscles, in contradistinction to the evidence presented by Kohstamm and Quensel (1908) and Tozer (1912).

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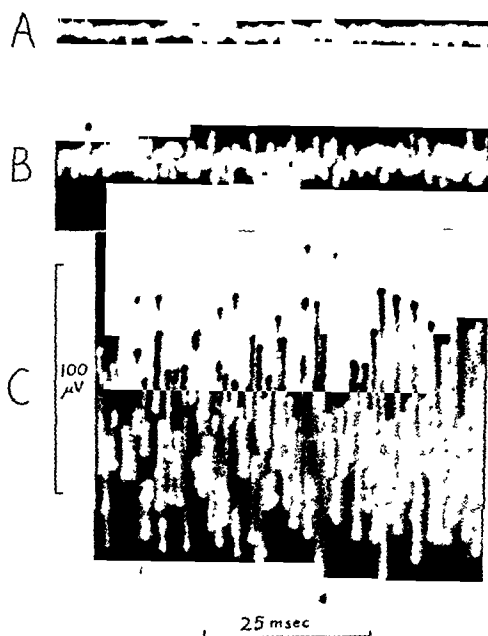


FIG. 1. Photographs of single sweeps of oscillograph. Velocity and amplification unchanged. Cat 83. A. Noise level of amplifier. B. Background activity of mesencephalic nucleus. Jaw closed. C. Activity recorded from the same point as record B when jaw was depressed. This represents a response recorded as 5+.

easily manipulated. A single 22 gauge nichrome wire, insulated with several coats of baked enamel except for a 0.5 to 1.0 mm. sharpened tip, was used as a unipolar lead, the machine itself being the other lead. (Potentials have also been recorded with a concentric electrode.) Action potentials were led through a 5 stage resistance coupled amplifier with a differential input (circuit of Toennies, 1938) to a cathode ray oscillograph and loud speaker. The entire brain stem in the region of the mesencephalic root and nucleus, from a level well rostral to the oculomotor nucleus to levels well caudal to the exit of the Vth nerve, was explored. The needle carrier of the Horsley-Clarke machine was tilted rostrally 35° so that the needle might pass to points caudal and ventral to the bony tentorium cerebelli without striking it (Fig. 2). This procedure avoided the necessity of removing the tentorium or entering caudal to it. The use of the Horsley-Clarke rotating needle carrier was described by Harrison (1938).

On the basis of stimulation and chromatolytic experiments, Lewy, Groff and Grant (1937, 1938) suggest that this nucleus gives rise to autonomic fibers passing peripherally with the three trigeminal divisions, and that stimulation of these autonomic fibers results in the pseudomotor response in degenerating facial and tongue muscle. Critical analysis of the findings of these workers will be reserved for the discussion.

There is no definite physiological evidence concerning the function of the mesencephalic root of the Vth cranial nerve. Considerable evidence gathered on the anatomical side suggests its logical rôle in the control of mastication. It has been the purpose of the experiments here reported to obtain irrefutable evidence regarding its function.

For this purpose the Horsley-Clarke stereotaxic instrument has been employed to place a unipolar lead within the mesencephalic root. Action potentials were led to a cathode ray oscillograph and loud speaker. The ease with which action potentials could be elicited from the mesencephalic root in response to jaw opening has been gratifying as have the selectivity and sensitivity of the response.

MATERIAL AND METHODS

Twenty adult cats were used in these experiments. Under light nembutal narcosis (30 mg./kg.) the Horsley-Clarke instrument was placed on the animal's head. The machine was suspended from a support in order that the jaw might be

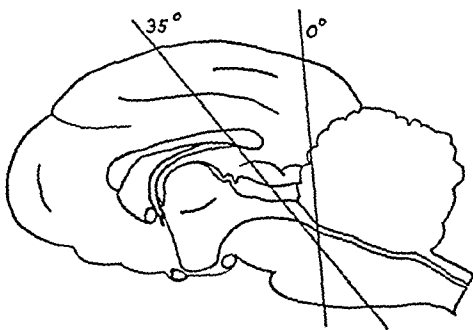


FIG. 2. Sketch of sagittal section of cat's brain. The line marked 0° indicates normal vertical plane of Horsley-Clarke machine. Line marked 35° indicates plane used in these experiments.

The technique consisted of inserting the needle into the brain along a line calculated to pass through the mesencephalic root. Stopping well above the predetermined root level, potentials were recorded before, during and after opening the jaw. A lucite or glass rod was used to depress the jaw. The needle was then projected 0.5 mm deeper, the jaw response recorded, and so on. In this manner the entire root was explored and its position mapped out. If no potentials were elicited through jaw opening, a new insertion of the lead was made 1 mm lateral or medial to the first one. We were usually successful in striking the root on the first puncture.

In 6 cats, the mandibular nerve was exposed extracranially just distal to its exit through the foramen ovale prior to the placing of the Horsley-Clarke machine on the cat's head. An electrode was then inserted into a rostral portion of the mesencephalic root, the insertion being halted when the first detectable impulses were elicited by jaw opening. The mandibular nerve was then transected and another attempt made to elicit responses from jaw opening. The entire rostro-caudal extent of the root was then explored, using the previously determined lateral coordinates, and testing for responses from passive movement of the eyes, from tactile stimulation of the skin of the head and the buccal mucosa, and especially from blunt pressure on the teeth and palate.

Using silver-silver chloride electrodes, responses have also been recorded from the inferior alveolar, superior alveolar, palatine, infraorbital, and ethmoidal nerves during blunt pressure stimulation of the teeth and gums, as well as tactile stimulation of the skin of the head, nasal and buccal mucosa.

Critical points, such as those from which exceptionally high potentials were obtained, were marked by placing a small electrolytic lesion at a point 1.5 mm lateral to the path of the unipolar electrode (Fig. 3, 4, 5 and 6).

In all experiments, following completion of the above procedures, the head of the animal was injected with 10 per cent formalin and the brain removed to formalin. On the following day, the brain stem was sectioned as nearly as possible in a plane parallel to the path of the unipolar electrodes, i.e., in a plane which lay at a 35° angle to the vertical plane of the Horsley-Clarke machine (Fig. 2). This angle of cutting accounts for the obliquity of the sections included in the illustrations. The brains were subsequently embedded in nitro cellulose, sectioned at 40 μ , every other section mounted and stained according to the Weil technique. All experimental results were then carefully checked in the microscopical preparations.

OBSERVATIONS

Action potentials recorded from within the central nervous system vary according to the neural structure with which the electrode is in contact, and with the physiological condition of the animal (Gerard *et al.*, 1936). As the electrode moves through the brain, injury potentials are elicited; these quickly disappear, leaving only the amplifier background plus the background of the particular region. The action potentials which have been elicited from the mesencephalic root of V by opening the jaw have been larger than any which have been specifically evoked from other portions of the brain stem incidentally studied in connection with this problem. When the electrode tip is actually within the mesencephalic root and the jaw is opened, the markedly heightened and more numerous action potentials are easily seen on the oscillographic screen (Fig. 1), and a roar is heard from the speaker. This response is prolonged throughout the period of jaw opening. Extension of the masticator muscles by gravity alone was sufficient to evoke an excellent response, while further extension gave rise to potentials as much as six times the height of the background activity (Fig. 1). The response to jaw extension was entirely abolished by passive closure of the jaw.

The best responses were obtained when the electrode was at its first position within the root and had not been withdrawn and reinserted. After

withdrawal, or projection and partial withdrawal to the original point, action potentials could still be elicited from jaw opening but were usually diminished in number and amplitude due to damage to the root.

The specificity of the response may be indicated by stating that on all occasions when the electrode passed approximately 0.5 mm. to either side of the root there was little response to jaw opening. Only a faint muffled roar was heard when the needle tip was this distance from the root. Such a response characterized proximity of the needle to the root, but was quite different from the response elicited from direct contact with it.

The response to jaw opening was maintained as long as the jaw was depressed and in that respect is in complete conformity with proprioceptive action potentials obtained elsewhere. Similar responses could be elicited by pressing on the homolateral masticator muscles or by pressing back the eyeball and thus indirectly pressing on the pterygoids (*sic*), a characteristic of the proprioceptors within striated muscle.

Because of the sensitivity and amplitude of the masticator stretch response, it is essential to eliminate this response before further study of mesencephalic root function. Both before and after section of the mandibular root, movement of the eyeball in any direction, and therefore stretch of the extrinsic ocular muscles, failed to produce potentials in any portion of the mesencephalic root. On the other hand, blunt pressure stimulation of the teeth of the homolateral half of the upper jaw, and especially of the homolateral canine tooth, resulted in a marked increase in the height and frequency of the action potentials obtained from the caudal half of the mesencephalic root (Fig. 6), in all 6 experiments in which the mandibular nerve was cut. This response may also be elicited from the teeth with the mandibular nerve intact, but the possibility of a response from jaw movement during stimulation is eliminated by section of the nerve. A response was also obtained from the homolateral hard palate, the most anterior portion adjacent to the canine teeth being the most sensitive region.

The canines were the most sensitive structures concerned in elicitation of potentials in the mesencephalic root following blunt pressure stimuli of oral regions. Pressure on these teeth in any direction resulted in a large burst of potentials from the caudal portions of the mesencephalic root (Fig. 6), lasting throughout the duration of the stimulus. Pressure on the gum or alveolar process overlying the canine tooth resulted in similar potentials. Pressure on the gums overlying the other homolateral teeth, sufficient to press on the underlying tooth roots, especially the incisors, evoked a response but to a lesser degree.

Except for the response resulting from jaw opening and the responses from blunt pressure stimulation of the teeth and hard palate, there were no responses from the mesencephalic root as the result of stimulation of other types from the head or buccal regions.

The mesencephalic root potentials are obtained solely from the homolateral masticator muscles, teeth and hard palate, never from contralateral

structures. Section of the mandibular division of V abolishes the jaw opening response in the mesencephalic root on the same side, leaving the response intact on the opposite side. Exploration of the mesencephalon at A4.0 (anterior Horsley-Clarke coordinate) failed to yield potentials from jaw movement. Histological examination of the brain stems at this level showed that the needle punctures were immediately rostral to the rostralmost portion of the mesencephalic root of V.

In all experiments, punctures made through the mesencephalic root from its rostral level (approximately A3.0) to the level at which the root turns

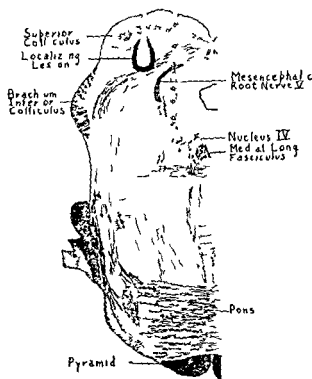


FIG 3 Projection drawing of section of brain stem of cat 4 in plane of angle 35°, Fig 2, at level of trochlear nucleus. The path of recording lead is indicated in dotted lines medial to localizing lesion. Numbers along path indicate intensity of response to depression of jaw. 3 indicates response of about 30 μ V. See text for additional description.

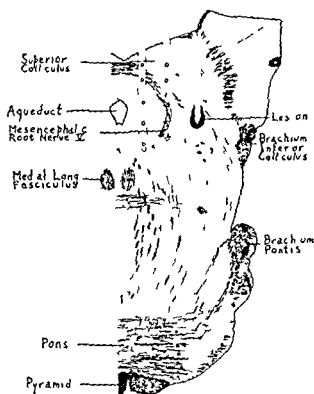


FIG 4 Projection drawing of section of brain stem of cat 55, at level 1 mm caudal to Fig 3. 5 indicates a response of greater than 50 μ V. Note complete absence of response to jaw opening from puncture medial to mesencephalic root.

laterally to enter the Vth nerve (approximately P7.0) gave rise to the characteristic response from opening of the jaw. The frequency of the action potentials was less from the rostral half of the root than from the caudal half, although the amplitude of the individual spikes was as great in one part as another. This decreased frequency of potentials in the rostral half is undoubtedly due to the diminished number of fibers in the root as it ascends in the mesencephalon.

When the electrode is inserted at or caudal to P8.0, a level histologically seen to be caudal to the laterally passing fibers of the mesencephalic root, no action potentials are obtained from opening of the jaw, or from pressure on the teeth or palate.

The selectivity and sensitivity of the responses from the mesencephalic root are well illustrated in the accompanying figures. All figures are actual line copies of projections of the brain stem (approximately $5\times$), the plane of section, as previously stated, being parallel to a plane at a 35° angle to the Horsley-Clarke vertical plane. The numbers within the electrode path indicate the absence (0) or presence (1 to 5, minimum to maximum response approximating $10\text{ }\mu\text{V}$ to over $50\text{ }\mu\text{V}$) of action potentials to opening of the jaw. In Fig. 6 the numbers within the electrode path indicate the response to blunt pressure stimulation of the teeth and palate. The lesion in each figure is lateral to the electrode point yielding the maximum response at that level.

Figure 3 represents a section at A1.0, or through that portion of the mesencephalic root lying at the level of the trochlear nucleus in cat 4. The electrode path traverses the dorso-medial portion of the mesencephalic root at the point marked 3. The response elicited at this point by jaw opening was of the intensity which we have arbitrarily designated as 3+ (about $30\text{ }\mu\text{V}$). It may be seen that the tip of the localizing lesion lies lateral to the maximum response. As the electrode passed ventrally, the response decreased and disappeared as the exposed tip left the root.

Figure 4 represents a section of the brain stem of cat 55 at A0.0, or 1 mm. caudal to the plane of Fig. 3. Here the electrode passed through most of the fibers comprising the mesencephalic root at this level. The number 5 (more than $50\text{ }\mu\text{V}$) indicates a maximum response at a point where the electrode tip was in direct contact with the main body of the root and lies directly medial to the localizing lesion. Note the complete absence of all response to jaw opening in the medial electrode path which misses the mesencephalic root.

Figure 5 represents a section just rostral to the decussation of the trochlear nerve. Here, as the electrode tip lay immediately lateral to the dorsal portion of the mesencephalic root, 1+ to 2+ (10 to $20\text{ }\mu\text{V}$) responses were obtained. Then, as the tip came to lie directly in the root medial to the localizing lesion, there were responses of maximal intensity (4+ to 5+). The response disappeared as the electrode passed ventral to the root.

Figure 6 represents a section through the opposite half of the brain stem of the same animal and at the same level as Fig. 5. On this side the mandibular nerve was sectioned before inserting the electrode at this level. While the electrode tip was medial to the lesion and within the mesencephalic root, action potentials were elicited from blunt pressure stimulation of the homolateral upper teeth and palate. A 3+ response was as large as was ever obtained from the teeth and then only by pressure on the homolateral canine tooth, the other homolateral teeth and palate giving a response of lesser magnitude. There was no response to jaw opening on this side after section of the mandibular nerve. Careful study of this illustration (and of the microscopical sections) shows that the needle was rostral to the level of the main sensory

nucleus of V, and almost 3 mm. medial to the position of this nucleus. Tactile stimulation of the palate, gums and other oral structures failed to elicit this response, although leads within the main sensory nucleus of V or the sensory root yield responses to such stimuli.

Leading from the inferior alveolar nerve, action potentials similar to those described for blunt pressure stimulation of the upper teeth are easily elicited by similar stimulation of the homolateral lower teeth, especially the canine. Tactile responses from the skin of the anterior portion of the lower jaw were also elicited from the inferior alveolar nerve, whereas tactile re-

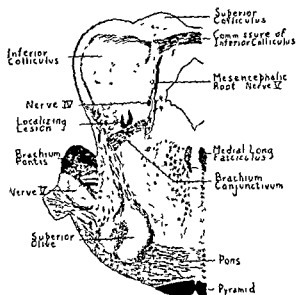


FIG. 5. Projection drawing of section of brain stem of cat 10 at level just rostral to trochlear decussation. Note that response to depression of jaw is 1 ($10 \mu V$) as needle lies immediately lateral to mesencephalic root and increases (3, 4 and 5; 30 to over $50 \mu V$) as needle enters root.

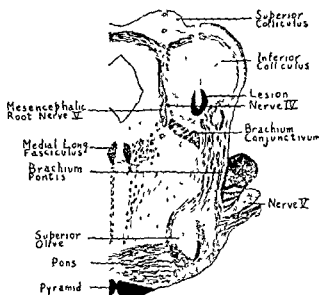


FIG. 6. Projection drawing of section of brain stem of cat 10, at same level but opposite side as Fig. 5. The mandibular nerve had been sectioned and responses noted were obtained from blunt pressure on upper teeth and hard palate. The response to depression of jaw was negative. A 3 represents maximum response obtained from stimulation of teeth and palate. Other data as in Fig. 3 and in text.

sponses were never obtained from the mesencephalic root. It is difficult to press on the teeth of the lower jaw without depressing the jaw and thus stretching the masticator muscles. No attempt, therefore, was made to record the effect of blunt pressure stimulation on the lower teeth from the mesencephalic root.

Leads from the superior alveolar and palatine nerves gave similar responses to blunt pressure stimulation of the upper teeth and palate, here again the canine being by far the most sensitive structure. Leads from the infraorbital nerves, central to the separation of the superior alveolars, gave in addition to the responses to blunt pressure stimulation of the teeth, tactile responses from the skin of the infraorbital region. We have been unable

to elicit potentials from the ethmoidal branch of the ophthalmic nerve on blunt pressure or tactile stimulation of the teeth, palate, snout skin or nasal mucosa.

DISCUSSION AND CONCLUSIONS

Action potentials have been elicited from the entire extent of the trigeminal mesencephalic root upon stretching the muscles of mastication. These responses have been identical with the proprioceptive impulses from peripheral nerve on stretching striated muscle (Mathews, 1933; Corbin and Harrison, 1938, 1939). They are slow to adapt, elicitable by pressure over the muscles concerned, and immediately abolished by section of the masticator nerves. Although the conduction rate of these impulses has not as yet been determined, the size of the potentials recorded indicates that we are dealing with fairly large fibers. Occasionally, on entering the mesencephalic root, we have elicited well spaced, regular potentials such as are obtained from single fiber preparations. Such individual potentials have always been large and undoubtedly mediated by single, large myelinated fibers.

Responses have been evoked when leading from the caudal half of the mesencephalic root from blunt pressure stimulation of the upper teeth and hard palate. Only lesions of the caudal half of the mesencephalic root and nucleus resulted in appreciable degeneration in the palatine and alveolar nerves, thus explaining our inability to obtain tooth and palate responses from the rostral half of the mesencephalic root (Corbin, 1940). These responses and those from jaw opening have been recorded from the mesencephalic root alone, not from immediately surrounding nervous tissues. Tactile stimulation of the skin of the head or buccal mucosa did not evoke responses from this root.

The area of distribution for the peripheral receptors for these responses corresponds precisely with the distribution of the mesencephalic root fibers as determined in degeneration experiments (*vide supra*). As previously mentioned, the histological studies demonstrated mesencephalic root fibers in the superior alveolar and palatine branches of the maxillary division of V as well as in the masticator and inferior alveolar branches of the mandibular division. Mesencephalic root fibers were also found in the ethmoidal branch of the ophthalmic nerve, but we have been unable in the work here reported to find any function for these fibers.

Action potentials have been obtained from the mesencephalic root fibers on jaw opening after these fibers have turned laterally and left the region of their nucleus, action potentials which were identical with those obtained from the ascending portion of the root. Primarily because of the evidence from degeneration experiments on this root (*vide supra*), it may be assumed that we have been recording from primary sensory neurons. The close similarity of the mesencephalic root potentials to the proprioceptive potentials which have been studied in the spinal accessory nerve substantiates this belief. However, one would have to determine accurately the conduction time to rule out the possibility of a post-synaptic component.

The responses here reported have been from the homolateral teeth, palate and masticator muscles, never from contralateral structures. This again is in complete agreement with our anatomical studies on the root, although Lewy, Groff and Grant (1938) suggest, on the basis of chromatolytic studies, that a goodly portion of the mesencephalic root V fibers cross to the opposite side. Corbin (1940) has discussed the evidence indicating that the receptors for deep pressure from the teeth are located in the periodontal membrane. Muscle spindles have been seen in the external pterygoid and masseter muscles of the rabbit (Cipollone, 1897) and in the masseter muscle of the fetal pig (Cuajunco, 1926). It is likely that similar endings in the masticator muscles of the cat give rise to the impulses which we have recorded in the mesencephalic root during stretch of these muscles.

The medullated fibers which arise from the medium to large sized cells of the mesencephalic nucleus of V may then be considered as forming the afferent limbs of masticator reflex arcs. The findings by Pfaffmann (1939) and Sherrington (1917) that mechanical stimulation of the maxillary nerve causes reflex jaw opening, and Sherrington's observations on decerebrate cats in which he demonstrated jaw opening from blunt pressure stimulation of the teeth, gums and palate, indicate that those mesencephalic root fibers arising from the periodontal membrane (especially of the canines in the cat) and palate mediate impulses which are inhibitory to the trigeminal motor nucleus cells. Acting in conjunction with these impulses are those arising from the muscles of mastication, the total mesencephalic root inflow thereby controlling and coordinating movements of the lower jaw, permitting a forceful bite without damage to the structures involved in mastication (*i.e.*, teeth, gums, and palate).

Bremer (1923) sectioned the mesencephalic root of V just rostral to the trigeminal motor nucleus in decerebrate cats and observed no decrease in tonicity of the masticator muscles in the majority of his animals. Because of this finding he concluded that the mesencephalic nucleus and root were not concerned in reflex activity of the masticator muscles. He admits that this evidence was from acute experiments lasting only a few hours, and that therefore such a section would not interrupt the afferent limb of the mesencephalic root to the motor nucleus of V, but would merely sever such fibers from their cells of origin. This leaves intact the mesencephalic root collaterals from the pseudo-unipolar cells of its nucleus to the motor nucleus of V. That such collaterals, severed from their cells of origin but retaining their peripheral connections, might maintain their functional activity for even a day or two is supported by the findings of Gibson (1940) who demonstrated maintenance of function in degenerating preganglionic cervical sympathetic fibers for two days following nerve section.

Rioch and Lambert (1934) found in 4 acute experiments and in one chronic experiment that section of the sensory root of V abolished the jaw-jerk and that there was complete flaccidity of the jaw closing muscles on the side of the lesion. These results are difficult to explain except on the basis of

possible damage to mesencephalic root fibers as they enter the brain stem.

We definitely feel, as a result of the morphological evidence reviewed and the physiological evidence of these experiments, that regardless of what other function or functions the mesencephalic root may be found to have, its fibers do mediate primary proprioceptive impulses from the muscles of mastication and deep pressure impulses from the teeth and hard palate.

The absence of responses in the mesencephalic root of V to types of stimulation of the head region other than the blunt pressure and stretch stimuli described offers another example of the central segregation of sensory functions, whereas the peripheral trigeminal branches contain sensory components of many types.

No action potentials could be evoked from any portion of the mesencephalic root of V as the result of passive movement of the eyeball; nor could any potentials of a sensory nature be obtained from leads placed directly on the oculomotor, trochlear and abducens nerves during extension of the extrinsic ocular muscles (unpublished data). This does not mean, however, that muscle sense fibers from the extrinsic ocular muscles may not have their cells of origin in the mesencephalic nucleus of V. It may well be that the sensory endings present in the extrinsic ocular muscles (Cilimbaris, 1910; Hines, 1931) do not respond to stretch, such as does the usual type of muscle spindle, but only to contraction of the muscles they innervate. We are at present attacking the problem with this possibility in mind. We can state now, however, that stretch of the extrinsic ocular muscles does not set up potentials which are of the usual proprioceptive type.

The results here reported lend no support to the findings of Lewy, Groff and Grant (1938), who believe that the mesencephalic root contains cells giving rise to cranial autonomic fibers which course peripherally with the branches of V and the stimulation of which leads to the pseudomotor phenomenon in the properly denervated striated musculature. Using the technique described in our experiments for accurately locating a stimulating electrode within the mesencephalic root no pseudomotor (Vulpian-Heidenhain) phenomenon could be elicited in chronically denervated tongue preparations, although this was easily obtained from stimulation of the reticular matter in the region of the VIIth nerve well caudal to the lowermost fibers of the mesencephalic root (Corbin, Harrison and Wiggington, work in progress). It may be assumed that the intense stimuli which Lewy, Groff and Grant believed they were applying to the mesencephalic root, actually spread to autonomic neurons located at lower levels.

The numerous theories regarding the function of the mesencephalic root and nucleus of V, advanced by early workers in neuroanatomy, may be dismissed for the most part without serious consideration. Castaldi (1926), on purely morphological grounds, has revived the theory that this root is motor in function. However, the majority of contemporary workers who have studied this nucleus histologically have concluded that the cells comprising it

closely resemble the sensory cells of the cerebrospinal ganglia (Allen, 1919; Clark, 1926; Weinberg, 1928; and Sheinin, 1930). It would seem that the physiological evidence presented in this paper must certainly rule out the possibility of a motor function for the majority of the cells in this nucleus. Furthermore, an admixture of sensory and motor neurons in the same nucleus, wholly contrary to the findings elsewhere in the central nervous system, would make it extremely unlikely that any motor cells, either autonomic or somatic, are located in the mesencephalic nucleus of the Vth cranial nerve.

SUMMARY

1. Using the Horsley-Clarke stereotaxic instrument for localization, action potentials in 20 cats have been picked up from the mesencephalic root of the Vth cranial nerve with a unipolar lead to a 5-stage resistance-coupled amplifier with a differential input and then to a cathode ray oscillograph and loud speaker.

2. Action potentials, characteristic of proprioceptive impulses elsewhere, have been elicited from all portions of the mesencephalic root in response to opening of the jaw and thence stretch of the masticator muscles. Careful histological study of the brain stems has demonstrated that this response was elicited only when the lead was within the homolateral mesencephalic root, never from surrounding neural structures or the contralateral root.

3. From the caudal half of the mesencephalic root action potentials have been elicited also from blunt pressure stimulation of the homolateral teeth and hard palate. In the cat, the canine teeth have been by far the most responsive of the oral structures.

4. The physiological evidence here presented demonstrates the function of those mesencephalic root fibers found in degeneration experiments to enter the alveolar and palatine nerves. Impulses traversing these fibers to the motor nucleus are probably chiefly inhibitory, preventing damage to the structures concerned in biting (gums, teeth and hard palate). These impulses and those passing over the masticator nerves from the muscles of mastication, mediated by the mesencephalic root fibers, constitute the afferent limbs of masticator reflex arcs, thereby coordinating and controlling chewing movements.

5. No action potentials have been elicited from the mesencephalic root of V or from the IIIId, IVth or VIth nerves as the result of stretch of the extrinsic ocular muscles. The authors realize that this evidence does not exclude the mesencephalic root from a role in the sensory innervation of the ocular muscles.

6. Absolutely no evidence has been obtained to support the assertion by other workers that the mesencephalic nucleus gives rise to cranial autonomic fibers passing to facial and tongue muscles. On the contrary, evidence is presented which indicates that an autonomic function for mesencephalic root fibers is highly improbable.

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OCULOMOTOR NERVE AND REFLEX DILATATION OF PUPIL

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IN 1883 Bechterew concluded that the dilatation of the pupil resulting from painful stimulation is caused solely by inhibition of the activity of the third nerve. Although since that time Anderson (1904), Gullberg, Olmsted and Wagman (1938), and others have maintained that the sympathetic also participates in reflex dilatation of the pupil they have not denied that inhibition of the oculomotor nerve plays the chief part. In 1939 Ury and Gellhorn, after section of the third nerve in the cat, found that "under normal

conditions the pupillary dilatation in response to pain is almost exclusively due to parasympathetic inhibition," while still more recently Ury and Oldberg (1940) concluded that the variability of the pupil to light or afferent stimuli is due wholly to variation in oculomotor tone.

For some months we have had under study 20 cats in which the oculomotor nerve was sectioned intracranially. Observations made after the local application of eserine tend to support the conclusion of Ury and Oldberg. The failure of many recent physiological texts to assign to the oculomotor nerve its proper place in reflex pupillary dilatation warrants publication of observations which, though in part not original, confirm the view that reflex dilatation of the pupil is primarily an inhibition of the third nerve.



FIG. 1. Cat no 2 four days after intradural section of the right oculomotor nerve. The pupil remained at this diameter (13 mm.) until the animal's death 4 months and 10 days after operation.

In the experiments to be reported one oculomotor nerve was sectioned intradurally through a temporal approach. In those instances in which the distal end of the nerve did not retract beyond the dura a short segment was removed from the proximal stump so that the two cut ends would not be left in apposition. Immediately after section of the nerve the pupil of that

side became dilated (Fig. 1). The dilatation was progressive until a permanent transverse diameter of about 13 mm. was attained. This maximal dilatation was not reached until recovery from the anesthetic. In cases in which ether was used it was accomplished within the hour, while with nembutal anesthesia it was not seen until the animal was fully awake some hours later. This difference apparently arises from a depressant effect of nembutal or other barbiturates on sympathetic tonus.*

The diameter of the pupil after third-nerve section remained 13 mm. throughout the period of study, which was from 2 to 6 months, except in two animals in which a narrowing subsequent to operation was proved at autopsy to be accompanied by gross and microscopic regeneration of the nerve. No change in the position or movements of the nictitating membrane was noted after oculomotor section. Although in some animals an apparent enophthalmos followed the operation it is believed that the operative deformity of the skull and the post-operative ptosis rendered this observation unreliable.

In 8 of the 20 animals in which the oculomotor nerve had been sectioned the sympathetic pathway to the iris was interrupted at a later date, either by sectioning the cervical sympathetic trunk or by removing the superior cervical ganglion or the stellate ganglion. Regardless of the method employed the pupil became narrowed to a new permanent diameter which varied from animal to animal within the range 7-10 mm. In cases in which the superior cervical ganglion or the stellate ganglion was removed this change was maintained indefinitely. In cases in which the operation consisted of section of the sympathetic trunk, regeneration after 3 or 4 weeks resulted in progressive enlargement of the pupil to the original diameter of 13 mm.

When only the oculomotor nerve was sectioned the pupil remained maximally dilated even though the animal was at rest. Such a pupil is not suitable for investigation of possible changes in sympathetic activity until it has been artificially constricted by addition of a miotic such as eserine. In every animal of this group it was found that when eserine was applied locally to the eyes there resulted a progressive constriction of both pupils. Without exception, however, throughout the period of advancing constriction the pupil on the side of the third-nerve section remained 1-2 mm. larger than the pupil of the normal eye, and when full eserine effect was obtained the pupil of the side operated upon measured approximately 2.5 mm. in transverse diameter whereas on the normal side the pupil measured only 1.5 mm. Thus the miosis produced by eserine was less in the parasympathectomized eye. With partial third-nerve regeneration, however, the normal response to eserine returned. An opposite modification was noted after

* That the drug exerts such an affect is evidenced by additional observations. The fully dilated parasympathectomized pupil could be reduced in diameter by inducing nembutal anesthesia. Moreover, under nembutal anesthesia the pupils were constricted and the nictitating membranes were protruded in normal animals, and in unilaterally sympathectomized animals sympathetic activity was depressed to such an extent that no difference in the response of the two pupils to the withdrawal of light could be observed.

the sympathetic was interrupted. In this case the eserine response was increased and this was true both in animals in which the parasympathetic path was intact and in those in which it had been severed. Our experiments shed no light on the nature of the diminished response to eserine following preganglionic parasympathectomy. With regard to the augmented response

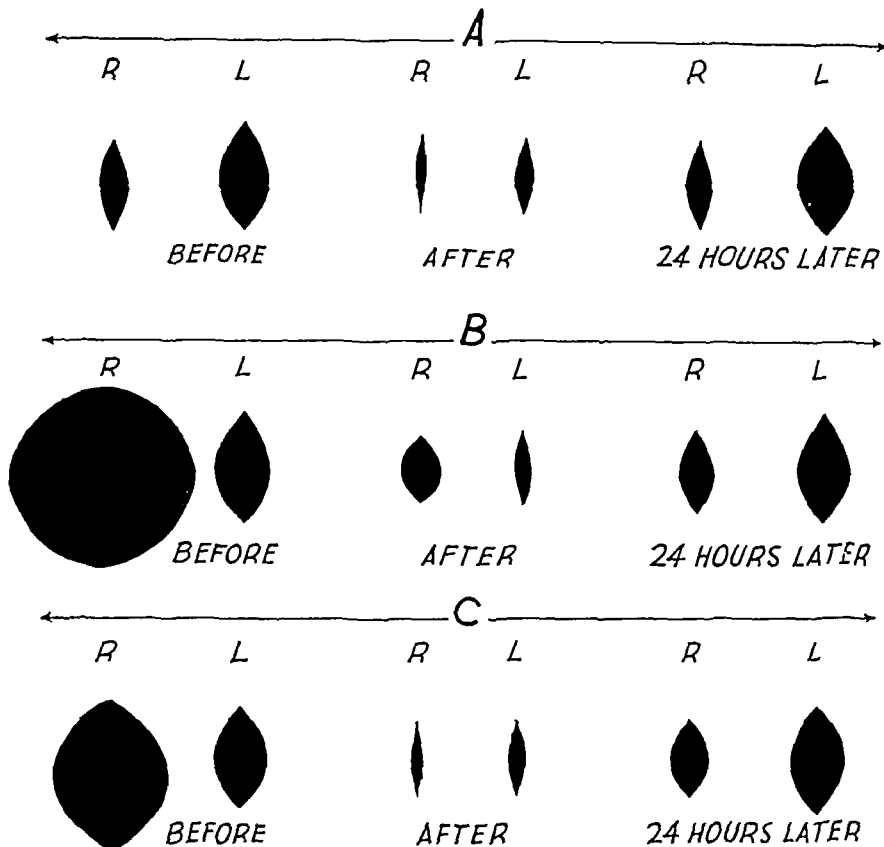


FIG. 2. Pupils before and after instillation of eserine into both eyes following (A) section of the right cervical sympathetic trunk, (B) section of right N. III, and (C) section of the right cervical sympathetic trunk and section of right N. III.

following sympathectomy it is reasonable to assume that it results from withdrawal of sympathetic dilator tone, which, when present, opposes eserine constriction.

Although the degree of constriction in response to eserine was decreased by parasympathectomy, the duration of the miotic effect was prolonged. Even after 24 hours, when the normal pupil had regained full activity, the parasympathectomized pupil still showed a decided constriction. This observation has been recorded previously (Anderson, 1905; Shen and Cannon, 1935). We have noted that subsequent sympathectomy did not alter this prolongation of the eserine effect.

These variations in response to eserine are shown diagrammatically in Fig. 2.

In a number of animals prepared by interruption of N. III and local application of eserine we attempted to evaluate the role of the sympathetic in pupillo-dilatation. Responses to the following stimuli were studied: (i) dark adaptation, *i.e.*, withdrawal of the normal light stimulus, (ii) painful stimulation such as electrical stimulation of the peri-anal skin or strong faradic stimulation applied directly to the sciatic nerve exposed under light ether anesthesia, and (iii) emotional excitement aroused by restraining the animal upon its back or exposing it to the barking of a dog. During the stages of partial eserization the pupil of the normal eye dilated instantly in response to each of the stimuli listed, but there was never the slightest visible dilatation of the pupil on the side of the third-nerve section.* After the eserine effect was complete the dilator response of the normally innervated iris was usually reduced and in some instances abolished. The sympathetic dilator path was still intact since both pupils responded readily to faradization of the cervical sympathetic trunks.

From these experiments it is apparent that inhibition of the activity of the third nerve constitutes the principal factor in the reflex pupillo-dilatation elicited by (i) withdrawal of light, (ii) painful stimulation, and (iii) emotional excitement. This conclusion was confirmed in another series of experiments in which the effects of these forms of stimulation were determined in 6 animals in which only the sympathetic pathway was interrupted. Observations made immediately following operation and after the elapse of some days were as follows:

(i) The sympathectomized pupil dilates readily on withdrawal of light. Under nembutal anesthesia no difference between the two pupils was observed in this respect. In the unanesthetized state, however, it was noted that while the response was prompt on the side of the operation the dilatation was not as great. Gullberg, Olmsted and Wagman (1938), using infra-red photography, noted that dilatation was prompt but slower in rate following sympathectomy and in some instances we have noted such retardation.

(ii) The sympathectomized pupil dilated readily in response to faradic stimulation of the sciatic nerve. The maximum diameter attained by the pupil on the side of the operation was never as great as that of the opposite pupil but the response was equally as prompt.

(iii) After sympathectomy the pupil underwent immediate dilatation in response to emotional excitement. Again, however, the dilatation was less than in the normal eye. An exception was noted in animals in which the superior cervical ganglion was removed. After an interval of 48 to 72 hours prolonged periods of emotional excitement led to augmentation of the dilatation. This augmentation, which appeared after some seconds, was much greater and more prolonged in the sympathectomized eye. It could be reproduced by the intravenous administration of 1 cc. of 1:100,000 adrenalin.

Therefore it was considered to be an adrenalin effect accompanied by sensitization on the side of the ganglionectomy. Thus, in addition to the immediate dilatation due to parasympathetic inhibition, prolonged emotional excitement gave rise to an adrenalin effect. The delayed adrenalin dilatation was distinguished easily from the prompt dilatation of third-nerve inhibition, for, in the latter case, periods of dilatation alternated rapidly with periods of constriction, the changes coinciding with momentary variations in the affective state.

Although Ury and Gellhorn (1939) concluded that pain stimuli elicit a dilatation of the pupil mainly by inhibiting the center of the third nerve, they stated, "What paths are involved in emotional excitement remains to be seen." That dilatation in response to emotional excitement is also effected through inhibition of the third nerve is evident, since this form of stimulation evokes immediate dilatation in the sympathectomized pupil but fails to do so when the oculomotor nerve has been cut.

SUMMARY

Observations upon the diameter of the pupil in the cat following pre-ganglionic parasympathectomy, pre- and postganglionic sympathectomy, and combinations of these procedures indicate that inhibition of the third nerve is responsible not only for the reflex dilatation elicited by withdrawal of light and by painful stimulation, but also for the immediate dilatation conditioned by emotional excitement.

The sympathetic and the parasympathetic are opposed in their action upon the pupil. Under ordinary conditions sympathetic dilator tone is remarkably constant while *third-nerve constriction is subject to extreme reflex modification*. Consequently changes in the size of the pupil depend upon reflex variations in the activity of the oculomotor nerve. That sympathectomy effects a diminution in the extent and the rate of reflex dilatation demonstrates that in normal animals the dilatation caused by inhibition of the sphincter pupillae is augmented by the tonic contraction of the dilator muscle.

The effects of eserine upon the normal, the sympathectomized, the parasympathectomized and the sympathectomized-parasympathectomized pupil are compared and are plotted in Fig. 2.

Observations are reported which lead to the conclusion that nembutal depresses sympathetic activity.

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MOVEMENTS ELICITED FROM PRECENTRAL GYRUS OF ADULT CHIMPANZEES BY STIMULATION WITH SINE WAVE CURRENTS*

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INTRODUCTION

TWENTY years after the classical paper of Leyton and Sherrington (1917) on the excitable cortex of the great apes was published, the task of stimulating the precentral gyri of three adult chimpanzees fell upon us. Although the chimpanzee has become a common laboratory animal, no recent attempt has been made to explore the whole precentral gyrus of this animal. Fulton and Keller (1932) reported results of occasional stimulation of this area (p. 97) in 7 chimpanzees (*Pan chimpanse*[=*satyrus*]) and Walker and Greene (1938) stimulated only the face area, in 4 animals (*Pan satyrus*). Leyton and Sherrington (1917) explored this gyrus in 22 chimpanzees (they mention two species, *Troglodytes niger* and *Troglodytes calvus*; Elliot's, 1913, synonyms, *Pan satyrus* and *Pan calvus*). Prior to that report all explorations of the "motor" cortex of this great ape were done in Sherrington's laboratory at Liverpool (Grünbaum and Sherrington, 1901, 11 animals; 1903, 5 animals; Graham Brown and Sherrington, 1912, 1913; and Graham Brown, 1914, 1916). The particular species used in those studies were also *Troglodytes niger* and *Troglodytes calvus*.

Three cytoarchitectonic studies of the distribution of Brodmann's areas 4 and 6 on the precentral gyrus of the chimpanzee have been published. First, that of Campbell (1905, p. 295) who studied one hemisphere each of two chimpanzees stimulated by Grünbaum and Sherrington in 1901 and 1902 (species given as *Anthropopithecus troglodytes*); second, that of Brodmann (1912) on one specimen of *Anthropopithecus troglodytes* (Elliot's, *Pan chimpanse*); and third, that of Bucy (1935) on 2 of the species, *Pan satyrus*, which had been used in Fulton's laboratory. Walker and Green related the results of their stimulation of the face area to its cytoarchitecture in 4 specimens of *Pan satyrus*. In each of these studies the distribution of areas 4 and 6 on the surface of the precentral gyrus was different. Whether this variation should be correlated with individual difference or with species difference or with the method used by the investigator cannot be determined at the present. Therefore, it becomes obligatory to correlate the results of electrical stimulation of each precentral gyrus with its own particular cytoarchitecture.

All of these investigators, with the exception of Walker and Green, used

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the faradic current from a laboratory inductorium as their source of electrical excitation. Since 1932 the present author has been using the sine wave current (see, Boynton and Hines, 1933; Hines and Boynton, 1940) for the exploration of the cerebral cortex of monkeys and common laboratory animals. Believing that the use of this current might uncover new facts not previously reported, these stimulations were undertaken.

MATERIAL AND METHOD

The three chimpanzees used in this study had been measured by Dr A. H. Schultz. Dayton, a male (*Pan leucopymnus*, Elliot, 1913, "all white") 15 years old, weighed 47.17 kg on the day of stimulation of his precentral gyrus (May 26, 1938). Evo, a female (*Pan Kooloo Kamba*, Elliot, 1913, "all black") 14 years old, weight 44.4 kg (February 18, 1938), and Mae, also a female (*Pan chimpanse*, Elliot, 1913, "white, with brown nose") weighed 44.9 kg (December 18, 1937). * They were therefore full grown adults. Each in turn was caught in a small room, anesthetized by an ether spray. A special table was constructed so that the trunk was supported and the extremities hung free. Ether was given throughout the duration of the exploration by tracheal cannula. The anaesthesia was deep, so that residual palpable tone was minimal. Thus the results were restricted almost entirely in all probability to activation of the cortico spinal tract.

Two types of exploration were done: (i) a general exploration of the whole precentral gyrus with the 60 c.p.s. sine wave current and (ii) an exploration of a single point with different frequencies of sine wave currents of 5 to 1440 c.p.s. The exploration of the precentral gyrus point by point should reveal the peculiarities of motor performance which characterize the chimpanzee, while that of a single point with various frequencies should establish the range of response of a small core of cortical neurones to electrical stimuli. The indifferent electrode was made of brass and kept in the rectum. The stigmatic electrode was a narrow platinum point (0.5 mm in diameter) mounted on a slender spring, which protected the surface of the cortex from undue pressure.

Maps of the cortical blood vessels were drawn upon celloid, a thin transparent tissue so pliable that it will easily cling to the cortical surface. Upon these maps, the points stimulated were recorded. In the case of chimpanzee M (December 18, 1937, Fig. 1) a transverse row of points placed anterior to the precentral fissure were stimulated initially; this row was followed by others placed on lines which cut this fissure at right angles. This method of stimulation was intended to eliminate facilitation. In the remaining two, E (February 18, 1938, Fig. 2) and D (May 26, 1938, Fig. 3) the initial point stimulated was placed on the crest of the central fissure. Stimulation proceeded rostrally point by point placed on imaginary lines cutting the precentral gyrus horizontal to the long axis of the cerebral hemispheres. The paracentral lobule was stimulated in M and D. E died before the dorsal crest of the precentral gyrus was reached. No attempt was made to explore the anterior wall of the central fissure.

Among the observers† were well qualified gross anatomists; consequently, the myology reported is, I hope, above reproach.

DATA

I. Results of exploration of precentral gyrus with 60 c.p.s. sine wave current

A. Area stimulated. If the tracing of the cortical surface of these three precentral gyri be placed on cross section paper, the area stimulated on the surface of M's brain was 816 sq. mm., of that of E, 692 sq. mm., and that of D, 918 sq. mm. The three topographi-

* Taxonomic study of chimpanzees since the time of Elliot's monograph on the primates (1913) indicates that the differing pigmentation of the face and extremities probably denote different racial groups rather than distinct species. All chimpanzees used hitherto in physiological work (save for Leyton and Sherrington's one specimen of *Pan calvus*, the bald head chimpanzee) belong to the species, *Pan satyrus*. The adult animals used in Dr Hines' study may be regarded as subspecies (Schwarz, *Ann. Mag. nat. Hist.*, 1934, 13 (10th ser.) 576) or varieties of *Pan satyrus* — Ed.

† The writer wishes to thank Dr. Lewis H. Weed, Dr. E. P. Boynton, Mr. H. C. Raven and Dr. T. R. Forbes, for their able and efficient collaboration during these stimulations.

cal areas of face, arm and leg in these three brains were respectively, 196, 372 and 248 sq. mm.; 161, 362 and 169 sq. mm.; 284, 245 and 389 sq. mm. The total number of points stimulated in *M* was 122 in *E*, 154; and in *D*, 312. The distribution of this number in the three topographical regions for each animal was respectively, in the face region, 28, 40, and 103; in the arm region, 49, 80, and 83; and in the leg area, 45, 34, and 126. Of those stimulated in the face area in *M*, 2 were silent; in *E*, 3; and in *D*, 13. In the arm area of each chimpanzee, 1 point was silent and in the leg area in *M*, 3 were silent; in *E*, 1; and in *D*, 19. In *D*, 3 points gave movements of both arm and leg.

The density of distribution of the points in the three topographical regions were as follows:—in *M* in the face area 1 point per 7.0 sq. mm.; in the arm area, 1 per 7.5 sq. mm.; and in the leg area, 1 per 5.5 sq. mm., giving an average 1 point per 6.6 sq. mm. In *E* the points were spaced more closely:—for the face, 1 per 4.0 sq. mm.; for the arm, 1 per 4.5 sq. mm.; and for that part of the leg area stimulated, 1 per 4.6 sq. mm.; giving an average spacing of 1 per 4.4 per sq. mm. In the case of *D*, the points were in each area more closely spaced than in *E*:—for the face area, 1 point per 2.7 sq. mm.; for the arm area, 1 per 2.9 per sq. mm.; and for the leg region, 1 per 3.0 per sq. mm., giving an average density of 1 point per 2.9 sq. mm.

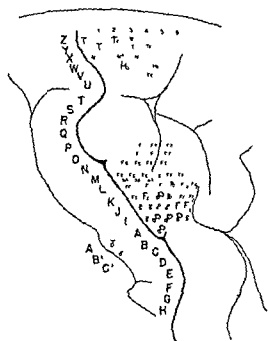
This spacing of points was closer than Leyton and Sherrington used if the numbers placed upon certain of the precentral gyri indicate the actual number of points stimulated: for example, by count 134 were stimulated on the right precentral gyrus and 96 on the left of chimpanzee No. 6. The writers had hoped that by placing the stimulated points closer together, a greater variety of movements would be elicited, and that evidence of cortical activation of individual muscles as well as muscle groups would be obtained.

B. Types of movement elicited. The movements elicited by stimulation of the precentral gyrus fell naturally into two categories (i) simple or isolated movements: those made by the contraction of a single group of muscles and, in some instances, of a single or part of a single muscle, and (ii) compound movements: those made by the contraction of more than one group of muscles.

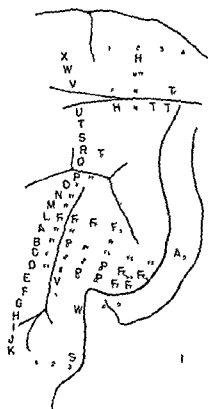
1. *Simple or isolated movements.* (a) *The face area.* Simple or isolated movements of muscles elicited by stimulation of the face area, innervated by the N. facialis, were as follows:—*M.* orbicularis oris (*M*, points F and I₂; *D*, pt. W₁₂), part of the *M.* platysma (*E*, pts. A₄, A₅, A₆, and E₁; *D*, pts. X₆ to X₁₀), *M.* risorius alone (*D*, pts. Y, Y₁, Y₄, Z, Z₁, a₃, and a₄), *M.* quadratus superior (*D*, pt. X₃) and the *M.* naso-frontalis (*D*, pt. W₆). Isolated movements of muscles innervated by the N. hypoglossus were obtained as follows:—*M.* longitudinalis superior (posterior fibers pt. I₃ in *M*, pts. b₁, e₃ in *D*; whole muscle, pts. C₂ to C₆, d, d₆, d₇, e, e₁, and h to h₃ in *D*, and pts. D and C₂ in *E*), vertical fibers of the tongue (pts. F₃, F₄, E in *E*; pt. f₃ in *D*), contralateral deviation of the tongue (pts. J, I in *M*, pt. F in *E*; pt. b₄ in *D*), slight contralateral movement of the tongue (pts. d₁ to d₅ in *D*), *M.* genioglossus (whole, pt. C in *E*, posterior part, pt. G₅ in *E*), and retraction of the tongue (pts. a₃, g₂, in *D*). Of the muscles innervated by the N. vagus, contraction of the contralateral *M.* glossopalatinus (pt. D₃ in *E*), of both *Mm.* palato-pharyngei (pts. b₂ and b₃ in *D*) was obtained. Contralateral deviation of the eyes (pt. F₁ in *M*), mastication (pt. G₁ in *M*) and opening of the mouth (pt. a₆ in *D*) were elicited only once each, and uncomplicated swallowing, twice only (pts. h₄, h₅, in *D*). Of the 90 reactive points in the face area of *D*, 48 give simple movements—11 of the 37 points in *E* and 7 of the 26 points in *M*. An increase in the number of points stimulated in the face area appeared to be related to an increase in the number of points giving single movements.

(b) *Remainder of precentral gyrus.* The reverse appeared in the case of the remainder of the precentral gyrus, for of the 189 reactive points in *D* only 91 gave isolated movements of a single muscle group; of the 112 in *E*, only 36; and of the 90 in *M*, only 40. Movements of the shoulder girdle itself occurred 17 times during the stimulation of the arm area of *D*, 3 times during that of *E* and twice in *M*. These movements were retraction, protraction, elevation and rotation (*D*), some form of adduction of the scapula (*E*), and abduction of the shoulder or contraction of trapezius giving retraction and elevation of the scapula (*M*).

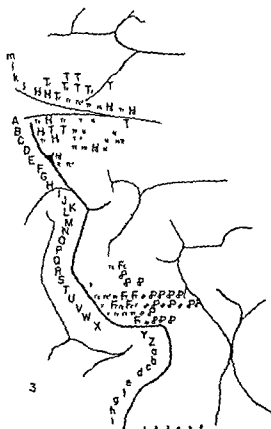
Movements of the upper arm alone were noted more frequently than those of the thigh alone, as 12 to 7. But peculiarly enough 8 of the 12 of the upper arm and 5 of the 7 of the thigh occurred in a single animal (*M*). The frequency of occurrence of certain of these movements is of interest, for retraction of the upper extremity was observed 6 times, protraction 2 times (*D* only) and protraction of the leg 27 and retraction 8 times. Internal



2



1



3

LEGEND

P	POLLEX ISOLATED	ADDUCTION
P		ABDUCTION
P ₁		FLEXION
P	PRIMARY	ADDUCTION
P		ABDUCTION
F ₁	FINGERS ISOLATED	FLEXION
F ₂		EXTENSION
"	PRIMARY	FLEXION
"		EXTENSION
"	ADDITIVE	EXTENSION
H	HALLUX ISOLATED	ADDUCTION
H		ABDUCTION
H ₁		FLEXION
H	PRIMARY	ADDUCTION
H		ABDUCTION
H	ADDITIVE	ADDUCTION
T ₁	TOES ISOLATED	FLEXION
T ₂		EXTENSION
"	PRIMARY	FLEXION
"		EXTENSION
"	ADDITIVE	EXTENSION
2, 3, 4, 5	DIGITS	

FIG. 1 to 3. These figures are pen and ink sketches of the cortical maps taken at the time of stimulation of each precentral gyrus. The legends are self-explanatory. Each dot marks the position of a reactive point. The first point stimulated is known by the letter of the row. The remaining points are identified consecutively by their number from the first point stimulated $\times 1$.

Fig. 1. Map of the left precentral gyrus of chimpanzee M.

Fig. 2. Map of the right precentral gyrus of chimpanzee E.

Fig. 3. Map of the right precentral gyrus of chimpanzee D.

and external rotation of the upper extremity was seen 3 times each, but external rotation of the lower extremity, 14 times; and internal rotation, 3 times. Abduction of the arm to abduction of the leg were as 8 to 2, and adduction of the thigh was noted once only (*M*). And in *D*, 5 separate points gave contraction of the anterior fibers of the *M. deltoideus*. Isolated flexion of the elbow was observed twice in *M* and 4 times in *E*; of the knee, 3 times in *D* and twice in *M*; extension of the knee, 6 times in *D*. Isolated flexion (3 pts.) and extension (2 pts.) of the ankle was obtained in *D*, but in both *E* (4 pts.) and *M* (1 pt.) only extension of the ankle. Similarly single extension (1 pt.) and flexion (1 pt.) of the wrist was found in *D*, and in *M* only flexion (1 pt.). Radial flexion of the wrist alone occurred in *D* only. No isolated supination or pronation was caused by stimulation of closely spaced points in the arm area of the male chimpanzee. Ten instances of simple pronation were noted in *E*, 2 in *M*, and but one instance of isolated supination in each of these animals. In all two points (*B*₆, *E*₁) on the cortex of *D* and one (*S*₂) on that of *M* caused isolated inversion of the ankle and three points (*C*₂, *B*₁, *J*₂) on the former cortical surface (*D*), isolated eversion.

The most frequently elicited movement of the fingers was adduction of the thumb (15 in *D*, 4 in *E*, and 3 in *M*). Isolated abduction of this member was obtained twice only (*E* and *M*). Simple adduction of the hallux occurred 6 times in *D* and twice in *M*, abduction twice only (*S* and *E*). A few isolated instances of movements of single fingers were produced, such as flexion of the index finger (*J*₅ in *E*; *D*₅ in *M*), adduction (*C*₅ in *M*) or flexion (*L*₂ in *M*) of the 5th finger. Isolated extension of the 5th toe was seen once (*I*₃ in *D*). Simple flexion of the 1st digit was also rare, occurring once of the thumb (*B*₁ in *M*) and once of the hallux (*C*₁ in *D*). Uncomplicated extension of the great toe was noted once only (*B* in *D*). Moreover, flexion of the lateral four fingers occurred more frequently than their extension, in ratios as follows:—6 to 1 in *D*, 2 to 1 in *E*, and 7 to 1 in *M*; and flexion of the lateral four toes to extension, in ratios of 9 to 1 in *D*; 2 to 0 in *E* and 4 to 0 in *M*.

The ratio of distribution of number of isolated movements of the lower extremity to that of the upper extremity was 43 to 48 (*D*), 8 to 28 (*E*, only part of leg area was explored), and 11 to 29 (*M*). Of these, the ratio of number of simple movements of digits to those of the proximal muscles elicited in the leg and in the arm areas were respectively as follows:—19 to 24, 22 to 26 (*D*); 4 to 4, 8 to 20 (*E*); and 4 to 7, 14 to 15 (*M*).

Therefore, the dominant isolated movements are retraction of the upper arm, protraction and external rotation of the thigh, flexion of the elbow, extension of the knee, pronation of the forearm, adduction of the first digits and flexion of the lateral 4. Isolated movements at the wrist and at the ankle did not show preference for any particular muscle group. Therefore, the 60 c.p.s. sine wave current can elicit contractions of single muscles or parts of muscles from the precentral gyrus of the adult chimpanzee.

(2) *Compound movements. Face area.* The compound movements which followed electrical stimulation of the face area fall naturally into three groups, namely: (i) those which result from the activation of more than one group of nuclei within a single motor nucleus, (ii) those which result from activation of more than one motor nucleus, and (iii) those which repeat a definite sequence of muscle contraction observed to be a part of the animal's reflex mechanisms.

Under the first group there are groups of muscles innervated by the *N. facialis*, the *N. hypoglossus*, and the *N. vagus*. Those innervated by the *N. facialis* were as follows:—retraction of both lips (*D*, *E*, and *M*), retraction of both lips and blink of the contralateral eyelid, retraction and depression of the lower lip, drawing in of the lower lip and closure of the contralateral eyelid (*M*), simultaneous contraction of *Mm. auricularis* and *zygomaticus*, of *Mm. quadratus superior* and *zygomaticus*, of *Mm. risorius* and *orbicularis oris*, of *Mm. orbicularis oris* and *quadratus superior* and of these two with the *auricularis group* (*D*).

Compound movements of the tongue produced by simultaneous innervation of more than one group of nuclei of the nucleus of the *N. hypoglossus* were observed, namely:—retraction and contralateral deviation of the tongue (*D*, *E*, and *M*), elevation, retraction, and contralateral deviation of that organ (*M*), elevation and retraction (*D* and *E*), elevation and contralateral deviation plus wrinkling, and protrusion and contralateral deviation (*E*).

Contraction of muscles innervated only by the *N. vagus* was a rare occurrence, for depression of the pharyngeal arches and of the soft palates was caused but once (pt. *g*, in *D*).

In general when two cramal motor nuclei were activated simultaneously these were the nucleus hypoglossus and either the nucleus facialis or the nucleus vagus. All three nuclei were sometimes innervated. Movements of the lips and tongue were of frequent occurrence, for example, retraction of the contralateral corner of the mouth and contralateral deviation of the tongue, mass retraction of the corner of the mouth or of the lower lip (*E*) with retraction of the tongue (*M*), or contralateral deviation of the tongue and retraction of the lower lip (*E*). Movements of the tongue and of the pharyngeal arches were frequently obtained, especially in the male chimpanzee—such as retraction or elevation of the tongue and depression of the contralateral pharyngeal arch, and elevation of the tongue and bilateral depression or contraction of the pharyngeal arches. Retraction of the lip, eversion of the tongue and closure of the mouth were elicited once (pt *I*, in *M*).

Contractions of the muscles of the contralateral ear were associated with those of the tongue (pt *b*₁, 0.28 mA in *D*), or with those of the lips (*mm* orbicularis, risorius, and auricularis, pt *Z*, at 0.28 mA in *D*), or with a mass retraction of the facial muscles in circular form (pt *F*₃, 0.6 mA, in *M*), and closure of the contralateral eye (pts *E*₁ and *E*₂, 0.6 mA in *M*) was associated with movements of the contralateral facial muscles.

The third class of compound movements produced by stimulation of this region were those which followed a certain sequence, often seen in the animal's use of these muscles or in its reflex patterns of sequential movement. Such movements were of lips and tongue, or of mouth and tongue, or of the muscles of the pharynx and larynx, with and without those of the tongue—*i.e.*, movements used in manipulation of the food within the buccal cavity or in deglutition, as follows—Closure of the lips followed by turning of the tongue to the right, eversion of the lower lip followed by retraction of the angle of the mouth, raising of the lower lip and deviation of the tongue to the right, elevation of the tongue and contralateral divergence followed by lowering of the soft palate and definite salivation, elevation of the uvula followed by swallowing (*M*), retraction and the lateral movement or elevation of the tongue followed by contraction of the palatine arches and elevation of the epiglottis, strong elevation and retraction of the epiglottis and marked contraction of the palatine arches, swallowing, followed by retraction of the tongue and closure of the mouth, and opening of the mouth and elevation of the tip of the tongue (*D*). Two rhythmical movements occurred when the face area of chimpanzee *E* was stimulated—retraction of the tongue and movements of the whole tongue followed by swallowing.

Arm and leg areas. For simplicity of analysis, movements elicited by stimulation of the arm and leg areas were classified as (i) those of the proximal muscles, *i.e.*, contractions of the muscles of the girdles or of those attached to the girdles, as (ii) those of muscles which move the knee or elbow, *i.e.*, of what shall be called the second joint, and as those which move the ankle or wrist, *i.e.*, of those which shall be called the third joint, as opposed (iii) to the twisting movements which supinate or pronate the lower arm or invert or evert the ankle, and as (iv) those which move the digits.

(i) *Proximal muscles.* Combinations of movements of shoulder girdle and of the upper arm seemed to follow certain patterns, such that elevation of the former was accompanied by retraction, internal rotation or abduction of the upper arm (*D* and *M*), retraction of the shoulder by adduction of the upper arm (*E*), and abduction of the shoulder by a circular movement of the upper arm (*M*). In one of these animals (*E*) the lower arm accompanied these movements: retraction of the shoulder was joined by flexion of the elbow and supination, and elevation by flexion of the elbow. In *D*, three consecutive points yielded retraction of the shoulder and contraction of the contralateral abdominal pressor muscles.

The combinations of movements of distal muscles with proximal muscles which followed upon electrical stimulation fell into two categories (a) those which began proximally and spread distally, and (b) those which began distally and spread proximally. Of the former, the most frequent type of co-innervation of the knee with protraction of the thigh was flexion (11 ×), for extension of that joint occurred only three times. With the rarer retraction of the thigh, extension of the knee was seen twice, and flexion 4 times. Flexion of the knee was the only movement to follow either external rotation (4 ×) or internal rotation (1 ×) of the thigh. Movement of distal muscles either accompanied or followed that of abduction or adduction of the thigh. Of the latter, in all responses of distally lying muscles which ended in some movement of the thigh, the included movement of the knee was flexion (4 in *M*, 7 in *E*, and 1 in *D*). Movements progressing from distal to proximal muscles, so frequently found to follow stimulation of the leg area, were seen but

twice in the total exploration of the arm areas of these three precentral gyri, as flexion of the fingers and elbow and internal rotation of the arm (pt. O_6 , in E) and as extension of the fingers, flexion of the wrist and elbow and elevation of the shoulder and an undetermined movement of the ear (pt. P_1 , in M).

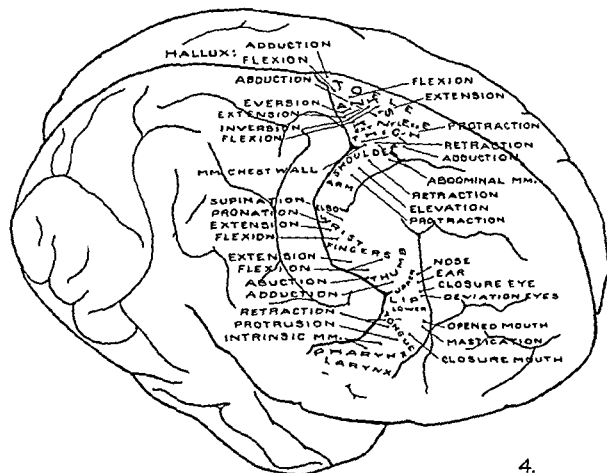
(ii) *Movements of second and third joints of the extremities either singly or as co-innervations* were produced from 41 points. However, co-innervations of these two joints only were relatively rare. Flexion of the wrist and of the elbow (pts. O_2 and N_2), and flexion of the ankle and of the knee (pt. T_3) were produced in M only; but extension of the ankle and flexion of the knee were obtained in both D (pts. M_5 and D_3) and in M (pt. T_2).

(iii) *Twisting movements.* The incidence of supination and pronation varied with the animal stimulated. In the co-innervations supination never occurred with extension of the elbow, only with flexion; and pronation only once with the former movement (M). Pronation was not obtained in a co-innervation which included movement of any muscle attached to the shoulder girdle; supination however was elicited twice as a part of movements involving such muscles in both E and M . The most frequently recurring movements with either supination or pronation were those of the fingers, extension or flexion. Such coinnervations might include or exclude movements of the wrist (either flexion or extension). Listing all combinations of movements of either pronators or supinators with those of the flexors or extensors of the fingers, in 22 movements out of a total of 25, the contractions of muscles of the fingers were primary in the sense of Leyton and Sherrington (1917).

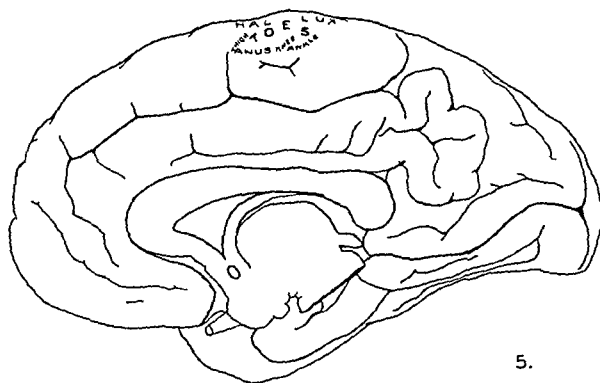
In the tabulation of movements of inversion and eversion of the ankle, the fact that the leg area of chimpanzee E was not completely explored may account for the lack of appearance of inversion of the ankle. In the remaining two animals 10 points (D) and 3 points (M) gave inversion of the ankle. All but 2 points (D_6 , C_7) in the former and 1 point (V_1) in the latter gave inversion as the initial movement. The additive movements obtained from all but 2 points (A_9 , C_7) in D and 1 point (V_1) in M were those of the toes or of the ankle. However of these 3 points in which inversion of the ankle was produced as an additive movement, points D_6 and C_7 (D) yielded movements of the toes as the initial response and point V_1 (M), protraction of the thigh. Points A_9 (D) gave flexion of the toes and of the knee as additive; point C_7 (D), not only inversion of the ankle but also extension of that joint, flexion of the knee and protraction of the hip; and point V_1 (M), not only inversion of the ankle but also flexion of the knee.

Of the 5 points which yielded eversion of the ankle, 4 gave that movement as primary. The movement elicited from this 5th point (F_1 in D) was retraction of the hip, flexion of the knee and eversion of the ankle. From 3 (C_8 , B_3 in D ; W in E) of the remaining points the additive movements were those of other muscles of the ankle or of the toes; but point U_5 (E) produced bilateral contraction of the abdominal musculature ($1 > r$) as the secondary movement.

(iv) *Digits.* Tabulation of the movements of the digits produced by electrical stimulation of the precentral gyri of these three chimpanzees allowed a few generalizations. First, the variety of elicited movements was so great, that aside from simple adduction of the first digits and aside from flexion of all or of the 4 lateral toes, exact duplication of particular movements was not observed. Duplication of particular movements occurred only 4 times each in the exploration of the cortices of M and of D , and twice, in that of E . Aside from adduction of the first digit or flexion of the remaining 4, 131 different movements were listed in which some movement of the digits was observed to be either isolated or primary. Of the primary movements of the digits the more frequently recurring ones were adduction of thumb and flexion of the fingers, and adduction of the hallux and flexion of the toes. Primary adduction of the thumb was elicited from 9 points in D , from 8 points in E and from 3 points in M ; primary adduction of the great toe, from 6 points in D and from 2 in M . Primary abduction of the thumb was obtained once each in D and M , and twice in E , while primary abduction of the great toe was even rarer, occurring once each in D and E and as a double movement of extension and abduction in M . Just as primary adduction of the first digit was elicited more frequently than primary abduction, so primary flexion of the lateral digits was caused more often than primary extension,—thus, ratios of primary flexion of the fingers to primary extension were as follows:—7 to 3 in D ; 15 to 11 in E ; and 9 to 6 in M . On the other hand primary flexion only of the toes was obtained, 3 times in D and E , and once in M . These movements of the digits occurred also as co-innervations. Adduction of the thumb was elicited with flexion of the fingers 19 times,



4.



5.

FIG. 4. Drawing of the lateral surface of the right cortex cerebri of a chimpanzee. The part of the body musculature responding to cortical stimulation is indicated as if projected upon the precentral gyrus. $\times 1$.

FIG. 5. Drawing of the mid-sagittal section of the brain of a chimpanzee, showing the part of the body musculature which responded when the paracentral lobule was stimulated. $\times 1$.

but with extension only once (pt. I_4 in E); abduction of the thumb, with extension of the fingers 3 times, with flexion twice. Similarly, adduction of the hallux was observed to occur with flexion of the toes 19 times, with extension, not even once; but with abduction of the hallux, flexion of the toes happened twice only and extension, 3 times. Therefore even in the compound movements abduction of the first digit was rarer than adduction and extension of the lateral 4 digits was rarer than their flexion. Also adduction of the first digit tended to occur more often with flexion of the lateral four; and abduction of the first, with extension of the lateral four digits.

The movements, secondary to primary flexion or to primary extension of the fingers, seemed to follow certain patterns. Although primary flexion or extension of the fingers was followed by extension or by flexion of the wrist, the proportion of occurrence of extension of the wrist to flexion of the wrist was greater when the primary movement was extension of the fingers than when it was flexion (7 to 5) and less when the primary movement was flexion of the fingers (5 to 11). The total number of primary flexions of the digits was 25 in all three cortices; of primary extensions, 18. Only once was the upper arm seen to participate in this group of additive movements (pt. O_6 in E).

Seven different combinations of movements were observed to follow primary flexion of the toes (all of the lateral 4). Among these additive (*i.e.*, any movement not primary) movements extension of the ankle was noted 5 times; flexion of the ankle, not at all; flexion of the knee, once only; and movements of the thigh on the hip twice,—once as a combined retraction and lateral rotation, and once as protraction, accompanied by contraction of the left abdominal musculature.

Movements of the digits also occurred as additive movements. For the first digits, such occurrences were relatively rare. Six such instances of additive adduction of the thumb followed primary flexion of the fingers, while the one instance of secondary abduction of the thumb was an example of deviation, beginning with pronation. No such complete uniformity was found when adduction of the hallux was elicited as additive, for movement of the ankle (extension or inversion) was noted in 6 of the 8 instances. These exceptions were flexion of the lateral 4 toes (pt. W_2 in M) and flexion of the knee (U_2 in M). Abduction of the hallux was seen among the movements elicited by stimulation of the precentral gyrus of D only. The primary movements here were extension of the 4 lateral toes, extension of the hallux and inversion of the ankle (2 points).

A comparison of the frequency of occurrence of secondary extension of fingers with that of secondary flexion shows that additive flexion of the fingers was definitely rarer than additive extension, for 14 instances of the former occurred and only 5 of the latter. The 5 different varieties of initial or primary movements which preceded extension of the fingers were distributed as follows:—extension ($2\times$) or flexion ($5\times$) or ulnar flexion ($1\times$) of the wrist, pronation of the forearm ($5\times$) and flexion of the elbow ($1\times$). In each of the 5 instances in which flexion of the fingers was secondary, the primary movement was different,—flexion of the elbow, flexion or extension of the wrist, contraction of the *M. brachii radialis* and pronation. The distribution of these secondary movements of the fingers in the various animals was respectively as follows:—extension 1 and flexion 3 in D ; 6 and 10 in E ; and 5 and 2 in M . On the other hand additive flexion of the toes occurred 7 times and additive extension, 5 times. Five of the former and all of the latter followed an initial movement of the ankle. The remaining two additive flexion of the toes followed primary protraction of the thigh or flexion of the knee.

Only certain of the co-innervations of thumb and of fingers were suggestive of use patterns. It is easy so to interpret adduction of the thumb and flexion of the lateral 3 fingers (pt. V_5 in D) or of the index finger (V_7 , V_8 in D) or of the index and 3rd finger (W_4 in D ; I_4 in E), or of the 3rd and 4th fingers (W_3 in D), or of apposition of the thumb to the fingers in flexion (K_3 in E) or to the 4th finger (L_4 in E). But serial flexions (2nd to 5th fingers, J_4 in E ; 2 to 4th, D_4 in M) or extensions (5th then 4th fingers C_5 , 3rd, 4th and 5th fingers, C_4 in M) of the fingers suggest not use patterns as such but rather the anatomical prerequisite for the ability to flex or extend the digits in different degrees in use. Similarly the simultaneous abduction of the thumb and extension of the 2nd finger (J_1 , at the 1st and 2nd joints J , M) could be so interpreted. There were only a few movements of the toes which could be considered cooperative, such as flexion of these toes and abduction of the hallux (J_3 , J_6 , D) and the reverse order (V_2 , V_3 , in M), flexion of toes (2 to 4) followed by that of the hallux (B_1 in D) or flexion of all 4 lateral toes plus flexion and adduction of the great toe (B_2 in D).

Again certain movements remained dominant, whether produced as isolated or compound. Retraction of the upper arm always exceeded protraction, external rotation of the thigh, internal rotation, pronation of the forearm, supination, and extension and inversion of the ankle, its flexion and eversion, respectively. Isolated or primary flexion of the fingers outran isolated or primary extension of the fingers, but additive flexion was rarer than additive extension. On the other hand certain movements were dominant when elicited either as isolated or as co innervations. Isolated retraction of the thigh was produced more often than isolated protraction, but the total number of protractions exceeded the total number of retractions. Isolated extension of the knee occurred more frequently than isolated flexion but co innervations including flexion of the knee outnumbered co innervations including extension.

Moreover, certain compound movements gave opportunity for interpretation of the capacity of the "motor" cortex, for contractions of some muscles tended to occur only in certain co innervations. Co innervations of the digital muscles found adduction of the first associated with flexion of the others, and abduction of the first, with extension of the remaining. Although either flexion or extension of the knee followed either retraction or protraction of the thigh, only flexion of the knee followed internal or external rotation. Supination and pronation were more often primary to contraction of proximally lying muscles, but inversion and eversion of the ankle to contraction of distally lying muscles (movements of the toes). However, when the first 3 of these movements were additive, the common initial movement was of the digits. As additive movements, flexion of the fingers was rarer than extension, but extension of the toes, rarer than their flexion. The spread of contraction from distal muscles to proximal muscles were more frequent and more extensive as responses to stimulation of the leg area than of the arm area. But the variety of the co innervations elicited from the arm area of the chimpanzee cortex was greater than those from the leg area.

C. Movements of extremities and of trunk not obtained. Only a few movements of the possible theoretical ones were not elicited by stimulation of the precentral gyrus of these three chimpanzees. No movements of the head on the neck, no extension at the elbow and no adduction of the arm were seen at any time. In *M* no radial or ulnar flexion nor extension of the wrist, no protraction or external rotation of the upper arm and no extension of the knee were obtained. In *E*, however, only protraction and internal rotation of the upper arm, flexion of the ankle, extension of the knee medial rotation and adduction of

of *D*'s cortex yielded from certain points the following —contraction of the abdominal pressor musculature on both sides followed by retraction of the left thigh (contralateral), flexion of the left knee and flexion of the right knee (pt *J*, 0.3 mA), retraction of the left thigh and abduction of the right thigh (pt *K*, 0.3 mA), contraction of the abdominal pressor musculature (pts *M*₁, 0.4 mA and *M*₂, 0.6 mA), protraction of right thigh, extension of the right knee and a slight tremor and undetermined movement in the left knee (pt *I*₁, 0.3 mA). Repeated stimulation of point *K* gave similar results, for with a similar strength of current and with a slight decrease (0.27 mA) a similar movement was obtained. But when the last strength of current was again applied, not only was retraction of the left thigh and abduction of the right obtained but slight retraction as well.

E's cortex proved particularly rich in bilateral responses of the abdominal musculature. The contraction of these muscles on the left was greater than the right and followed or preceded eversion of the foot (pt *U*₅, 1.5 mA), extension of the ankle (pt *W*₃, 1.0 mA), protraction of the leg, retraction of the arm (pt *T*₅, 2.0 mA), and protraction leg, flexion knee, extension ankle, spreading of toes and external rotation and abduction of the upper arm (pt *U*₁, 1.0 mA).

(b) *Unusual responses.* Changes in respiratory movements were observed in all 3 animals. Sudden and tremendous over-ventilation of the lungs followed immediately upon the stimulation of two points (*a*₅, 0.27 mA, and *i*₆, 0.6 mA) anterior to the inferior precentral fissure and anterior rostrally placed on the precentral gyrus of *D*. The other changes in respiration appeared to accompany movements of the chest wall itself and were located near the leg arm interregional border. Point *K*₁ (0.27 mA) on *D*'s precentral gyrus gave a change in respiration by contracting the anterior wall of the thoracic cage, and the first stimulation like that of *K*₁, of point *X*₄ (*E*'s cortex) elicited a deepening of the respira-

tory movements after a slight inhibition. A second stimulation of this same point (current strength similar, 2.0 mA.) added, to this delay and deepening of the respiratory movements, protraction of the thigh, flexion of the knee, and extension of the ankle. In *M* the initial stimulation of point O_3 (0.6 mA.) produced abduction of the shoulder girdle. Repetition of stimulation of this point (0.8 mA.) was followed not only by abduction of the shoulder and circumduction, but also by a respiratory spasm.

Vocalization was obtained as a single result upon the second stimulation of point E_1 (0.8 mA.) in *M*, and a light whistle followed wrinkling of the lips in the midline and that of the corner of the mouth when point G_3 (0.6 mA.) was touched with 0.6 mA. of the sine wave current. In this same animal the second stimulation of point K_3 (0.4 mA.) caused not only an elevation and deviation of the tongue to the right, and a lowering of the soft palate, but also an increase in salivation.

Well within the face area of *E*'s precentral gyrus, stimulation of point C_1 (1.0 mA.) yielded not only deviation of the tongue to the left and slight retraction of the lower lip, but also retraction of the contralateral arm accompanied by flexion of the wrist.

E. Localization of points on precentral gyrus from which movements were elicited. The three topographical regions were definitely outlined on the surface of the precentral gyrus of each of these three chimpanzees. A line drawn from the caudal spur of the superior precentral fissure cutting the central fissure through its dorsal rostral curvature formed the interregional leg-arm boundary. A ventrally placed line cutting the inferior precentral fissure and the central fissure about the middle of its sharpest ventro-rostral curvature separated the arm and face regions. On *M*'s cortex these two lines were parallel to the horizontal axis of the cerebral hemispheres. On both *E*'s and *D*'s cortices these separating lines were irregular.

Within the face area stimulated in these three brains the greatest surface responded with tongue movements and the least, with muscles innervated by the tenth cranial nerve (nucleus ambiguus portion). The area which yielded movements of the facial muscles could be divided into 3 zones,—a dorsal for movements of the upper lip, a ventral for those of the lower lip, and a rostral for movements of the muscles of the nose, ear and eyelid. Movements of the tongue musculature were obtained in three fairly distinct concentric zones running across the precentral gyrus, dorso-rostrally to ventro-caudally, giving respectively, retraction, protrusion and movements of intrinsic muscles. Anteriorly, both opening and closure of the mouth were located. Such a cortical pattern suggests direct neuronal relationships with individual nuclei of the nucleus facialis and the nucleus hypoglossus.

Within the arm area the most extensive surface was assigned to movements of the thumb. The thumb area adjoined the face area on its dorsal border. The points which gave isolated or primary movements of the flexors of the fingers were found close in cortical space to those which responded with movements of the thumb. In general, the flexor points were located more ventral and rostral on concentric arcs than were those which yielded isolated or primary movements of extension of the fingers. No exact localization for the movements of individual fingers could be discovered in these results. However, instead of the ladder-like representation of the digits given for the human (Foerster, 1936), movements of the 5th finger and those of the 2nd were sometimes elicited from neighboring points. Reading the movements produced from points which lie on the caudal division of the precentral gyrus, the somatotopical distribution was substantiated, for passing from the more ventrally to more dorsally lying points the muscles responding climbed the arm. However, if the movements caused by stimulating the more rostral division of the precentral gyrus be projected upon the points from which they were obtained, it was discovered that about midway on the rostral border the representation of the more proximal muscles curves ventrally to meet that of the more distal muscles curving dorsally.

In the leg area abduction of the hallux was observed as a frequent co-innervation with extension of the toes, adduction with flexion. But adduction of the hallux and flexion of the toes were frequently produced separately. The points from which the former movements were obtained were located more ventrally and on the lateral surface of the precentral gyrus. The latter movements were elicited from more dorsally lying points on the precentral gyrus or from points on the paracentral lobule. In *E*, no isolated or primary adduction of the hallux was obtained, only abduction of that digit. The area which in the other two cortices yielded isolated adduction of the hallux was not stimulated. In the other two cortices, the surface area which gave primary or isolated adduction of the hallux or

flexion of the toes was greater than that which gave abduction or extension. Aside from this particular localization of extension and flexion of the digits, the proximal muscles of the leg were localized more ventrally on the lateral surface of the precentral gyrus than were the distal muscles. However, the most rostrally lying points brought the representation of the distal muscles closer to that of the proximal muscles.

On the paracentral lobule of *M* all movements of the toes were those of flexion of *D*, 10 of the 29 reactive points gave flexion of the toes, and 2, extension. Seven points (*D*) gave adduction of the hallux and 1 point, abduction. Three points of the 18 reactive ones found on this part of the motor cortex of *M* gave adduction of the hallux. Flexion of the knee and protraction of the thigh, extension and inversion of the ankle were elicited from both, but eversion from *M* only and internal rotation of the thigh and flexion of the ankle from *D* only. Contraction of the anal sphincter was obtained from the more rostral and ventral points of this lobule in both. In *M* this movement occurred also as a co innervation, with extension of this ankle, in *D*, as a co innervation with protraction of the thigh. However, in *D* the latter movement also occurred from the two most ventro rostral points as a co innervation with contraction of the levator ani.

Movements which involved muscles acting at 3 or more joints and produced by threshold stimuli were located on the anterior border of the arm or leg regions. Similar movements, however, were caused by supraliminal stimuli of more caudally lying points.

The bilateral movements observed in *D* and *E* were all obtained by the stimulation of points lying in the vicinity of the leg arm inter regional borders.

No ladder like representation of the digits on the precentral gyrus was uncovered in any one of these stimulations. As noted in the previous section, the only movements of individual digits found, were those of the index and little fingers. Such movements were obtained from points as widely separated as the whole width of the "motor" cortex itself. For example, flexion of the index finger was elicited from point *D*₁ in *M* (caudal), from points *J*₈, *K*₈, *L*₈ and *L*₈ (rostral) in *E*, and flexion of the 5th finger, from *L*₃ (midway) in *M*, and from *L* (caudal) in *E*.

The change in respiration which could be allocated to changes in rhythm of movement of the chest wall were found in *E* (pt. *X*₄) rostrally in the leg area, and in *D* (pt. *K*₁) caudally in the leg area. The over ventilation of the lungs obtained twice in *D* was found outside the precentral gyrus (pt. *a*₃) and on the rostral border (pt. *i*₄). The vocalization (pt. *E*₁) and the light whistle (*G*₁) elicited in *M* were obtained dorsally on the face area.

F Question of threshold of "motor" cortex. Projection of the thresholds of the points stimulated upon each cortex in turn showed, (i) that whatever the average threshold for the particular cortex, the greatest number of low threshold points were found in the face area, (ii) that a particular cortex appeared consistent, maintaining throughout the experiment a characteristic threshold, and (iii) the greater number of points having similar thresholds were found to cluster about similar values for both the leg and arm areas in each animal.

Face area. On *E*'s face area 26 out of 37 reactive points had a threshold of 0.7 mA, on *M*'s face area, 5 points had a threshold of 0.4 mA, 7 of 0.6 mA, out of 26 reactive points, and on *D*'s face area, of the 90 reactive points, 21 responded to 0.22 mA of current, 28 to 0.28 mA, and 21 to 0.36 mA.

Arm area. Similarly in the arm area of these respective cortices, 31 points yielded movement to 0.7 mA of current and 36 to 1.0 mA of the 79 reactive points on *E*'s cortex, 17 points to 0.6 mA and 11 to 0.8 mA out of 48 reactive points stimulated in this region of *M*'s precentral gyrus, and 35 points to 0.36 mA and 13 to 0.4 mA out of 82 reactive points in the upper extremity area of *D*.

Leg area. Of the 33 reactive points stimulated on the leg area of *E*, 10 yielded movements to 0.7 mA of the 60 c.p.s. sine wave current and 17 to 1.0 mA, of the 42 reactive points on this region of *M*'s cortex, 10 gave movements when stimulated with 0.6 mA and 12 with 0.8 mA, and of the 104 reactive points on *D*'s leg area, 30 points responded to 0.36 mA and 16 points to 0.4 mA.

The thresholds of points which were lower than the above had the following values and distribution—for *E*'s face area, 1 point at 0.5 mA, for *M*'s face area 3 points at 0.2 mA, for her arm area 2 points at 0.3 mA, 6 at 0.4 mA, and 2 at 0.5 mA, and for her leg area, 2 points 0.3 mA, and 4 at 0.4 mA, and for *D*'s face area, 21 points at 0.22 mA, for his arm area 2 points at 0.22 and 0.24 mA each and 8 at 0.32 mA, and for his leg area, 2 points at 0.27 mA, 17 points at 0.3 mA, and 4 at 0.33 mA. The low threshold points on

the face area yielded movements of single muscles about the mouth or of the tongue. All of these points except certain ones in *D* (12 points *i.e.*, pts. X_6 to X_{10} and pts. W_2 to W_{12}) were found caudally on the gyrus in the dorsal region of the face area. The low threshold points of the arm area were also located caudally in the precentral gyrus of *E* and of *M*. All of these low threshold points, except 4 (pts. V_8 to V_{11} , 0.32 mA.) which were located ventrally and rostrally, were also distributed caudally on *D*'s arm area as well. The low threshold points on *D*'s arm area yielded either isolated or primary movements of the fingers and of those on *M*'s middle precentral gyrus, 6 out of 10 had caused movements either of the thumb or of the fingers. In the leg area the low threshold points were found distributed on the caudal part of the precentral gyrus. However, the movements which these particular points produced were frequently (not always) isolated movements of a part of the musculature of the lower extremity; but unlike the low threshold points of the arm area which yielded movement of distal muscles, those of this leg were unconfined to particularly placed muscles.

The high threshold points in the face area were located on the anterior border in *E* (g_5 , 1.5 mA.) beyond the inferior precentral fissure in *M* (1.6 mA., pts. I, J, K; 2.3 mA., pts. H, G, F, E) and either on the anterior (0.6 mA., pt. W_6 ; 0.48 mA., pts. X_5 and Z_6 ; 0.4 mA., pts. g_6 and g_7) or on the ventral (0.6 mA. pts. i to i₆; 0.4 mA., pts. h to h₅) border in *D*. Again without exception the high threshold points in the arm areas of these three cortices were discovered on the anterior border or on the rostral portion of the precentral gyrus (in *E*, 1.5 mA., pts. K_5 , M_6 , O_5 , O_6 , Q_7 ; 2.0 mA. pts. N_4 , P_6 , R_5 , T_5 ; in *M*, 1.2 mA., pts. D, N, O; 1.4 mA. pt. L and 1.8 mA., pt. M; in *D*, 0.8 mA., pt. T_5 , R_4 to R_6 , P_4 , N_4 ; 0.6 mA., pts. S_4 , P_3 , M_5). The high threshold points of the leg area were localized in all three chimpanzees on the rostral border of the precentral gyrus and in *M* and *D* on the ventral border of the paracentral lobule as well (in *E*, 1.2 mA., pt. Y_4 ; 1.5 mA., pts. U_5 , U_6 , Z_6 ; and 2.0 mA., pts. X_4 and W_4 ; in *M*, 1.2 mA., pts. Q, T, U, V, W, X, X_1 ; and 2.0 mA., pt. X_2 ; and in *D*, 0.8 mA., pts. E_4 , A_5 , M_3 to M_{11} , l_{10} ; 0.9 mA., pts. I_4 , H_5 , G_5 , F_5 ; and 1.0 mA., pts. D_7 , C_7).

In general then, the elicitation of movement from the more anterior edge of the precentral gyrus required a stronger current than from points lying 1 or 2 mm. posteriorly. This was found to be true whether the initial order of stimulation was transversely across the rostral crest of the inferior precentral fissure as in *M*, or whether each row of points was explored separately beginning on the anterior crest of the central fissure as in *D* and *E*. Although in the latter two cortices facilitation must have played a part, nevertheless, the abruptness with which the threshold was raised on the anterior border of the gyrus appeared to indicate that the edge of area 4 had been reached.

No generalization can be made about the type of movement elicited from these several high threshold points, except to say that the movements were rarely simple isolated movements at single joints. Often these movements were widespread co-innervations involving synergic groups of muscles located either proximally or distally or throughout a single extremity.

G. *Reversal; deviation; facilitation.* Of the 153 points (total all 3 cortices) restimulated with an intensity of current at threshold or above, only 2 points (K_2 and S_2 in *M*'s cortex) showed deviation of response. Stimulation of K_2 with 0.4 mA. elicited a ripple divergence of the tongue to the right; repetition (no change in current strength) elicited elevation of the uvula and narrowing of the fauces. Point S_2 yielded on 1st stimulation (0.8 mA.) contraction of the hamstring muscles; on 2nd (1.0 mA.), contraction of the *M. biceps femoris*; and on 3rd (1.2 mA.), inversion of the foot. Simple reversal, although not as infrequent, was also rare. In these three cortices the reversal common to all was that of abduction or adduction of the first digit. On *E*'s cortex, 0.7 mA. caused abduction of the thumb (pt. I_3); followed by 1.0 mA. the stimulation gave adduction of the thumb. Stimulation of point D_2 in *M*'s cortex with (i) 0.6 mA. yielded abduction of the thumb, (ii) 0.4 mA. gave no movement, and (iii) 0.5 mA. adduction of the thumb. In this same animal 0.6 mA. current elicited, from point U_1 , flexion and adduction of the hallux, and 0.4 mA., extension and abduction of the hallux. The initial stimulus of point A, (chimpanzee *D*) produced adduction of the hallux (0.6 mA.); the 2nd stimulus (0.4 mA.) gave abduction followed by adduction.

Facilitation was obtained with great ease in all 3 cortices with a series of stimuli of increasing intensity. In the case of *M*, 3 points yielded facilitation when stimulated with a series of decreasing intensities of current. One example will suffice. Point S_1 gave

(i) with 0.8 mA of current, flexion of the knee and protraction of the thigh, (ii) with 0.6 mA, the same movement plus lateral rotation of the thigh, and (iii) with 0.4 mA, the movement elicited with 0.6 mA plus extension of the foot

Centripetal individuation On the other hand stimulation of a single point (all 3 cortices) with a series of decreasing strengths of current elicited a decrease in the number of muscles contracting or in the number of fibers within a given muscle. Two examples will suffice to illustrate this phenomenon which Boynton and Hines described in 1933 (i) With 0.32 mA of current applied to point W_3 (chimpanzee *D*) there resulted flexion of the 4 lateral fingers, adduction of the thumb, flexion of the wrist, and supination of the forearm (ii) with 0.28 mA, adduction of the thumb and flexion of the 3rd and 4th fingers, and (iii) with 0.22 mA, adduction of the thumb and slight flexion of these fingers. Point S_2 (chimpanzee *M*) when stimulated (i) with 0.8 mA produced protraction of the thigh and flexion of the knee, (ii) with 0.6 mA, slight flexion of the knee, and (iii) with 0.4 mA, a twitch of the hamstring muscles, not sufficient to move the knee

The occurrence of these 4 phenomena will be discussed further when the results of stimulation of single points with different frequencies of the sine wave current are presented

H Results of stimulation of points outside precentral gyrus (a) *The postcentral gyrus* In order to determine whether or not stimulation with this current required the process of facilitation to make the postcentral gyrus reactive, the first point on *E*'s cortex touched with the stigmatic electrode was located immediately anterior to the inferior limb of the fissura postcentralis on the level of point *A* as given in precentral gyrus. This point *A'* (Fig. 2) yielded adduction of the thumb (1.0 mA). The next point below *A'*, *B'*, gave adduction of the thumb, flexion of the wrist, supination and slight flexion at the elbow (1.0 mA). Strangely, point *A'*, placed 2 mm anterior to *A'* gave nothing even with 1.5 or with 2.0 mA, nor did point *C'*, 2 mm ventral to *B'* with 1.2 mA

After the exploration of the whole of the precentral gyrus and of one point with certain frequencies, a point opposite *A*, on the precentral gyrus of *M* was stimulated on the postcentral gyrus, point *A'*. Point *A*, had not been stimulated for 4 hours and 45 min. Point *A*, gave (2.0 mA) flexion of the fingers and pronation

(b) *Area 6* All points initially stimulated in *M*'s cortex from point *A* through point *O* lay on the anterior crest of the inferior precentral fissure. The results of these stimulations were mentioned above

After the complete exploration of *M*'s "motor" cortex and of one point (*D*₂) with various frequencies of the sine wave current, 3 points widely separated but well within area 6 were stimulated. Point 6A (2.0 mA), gave a slow clonic protraction of the thigh, point 6B (2.0 mA), a slow clonic protraction of the thigh, and point 6C (1.0 mA), flexion at the knee, protraction of the thigh and extension of the ankle. This movement was of wide amplitude and clonic. Restimulation of this point with 2.0 mA added flexion of the toes and changed the clonic aspect of the movement to that of a beating movement

At a similar period in the experiment and over a similar area, 7 points were stimulated in *D*'s area 6, the first 4 points with 1.0 mA of the 60 c p s sine wave current and the last with 0.8 mA. These movements were as follows—points 6a and 6b a jerky protraction of the thigh, 6c, a similar movement but followed by repeated tonic contractions of the protractors, 6d, a jerky protraction of the thigh and flexion of the knee, 6e, repeated with 0.8 mA yielded retraction at the shoulder and flexion of the elbow, 6f, retraction and external rotation at the shoulder and slight flexion of the elbow, and 6g, external rotation of the upper arm

II Results of stimulation of single point with different frequencies (5 c p s to 1440 c p s) of sine wave current (Fig. 6)

After completion of the exploration of the surface of the precentral gyrus with the 60 c p s sine wave current, point *D*₂ on that of *M* and point *C* on that of *D* were chosen for exploration with various frequencies of the sine wave current ranging from 5 to 1440 c p s. The order of stimulation of these two points with these frequencies was similar. The general level of the intensities of the 60 c p s sine wave current found luminal for the surface of the "motor" cortex of *M* was higher than that for this portion of *D*'s cortex. Similarly, the effective strength of the frequencies applied to point *D*₂ on *M*'s cortex was higher than the effective intensities of similar frequencies applied to point *C* on *D*'s cortex. This minimum threshold frequency may be called the optimum frequency. Plotting the threshold for movement against the logarithm of the frequency produced the curve in

Fig. 6. The gradient of the curve for frequencies below the optimum frequency was steeper than that for those above. For both point D_2 and point C on these two cortices the optimum frequency (of those used) was 90 c.p.s. (0.13 mA., pt. C: 0.25, pt. D_2). In each exploration the frequencies which approached the minimum effective intensity of the optimum frequency were 120, 180, and 240 c.p.s. (0.2 mA., for each of these 3 for pt. C: 0.4 mA., for the 1st. 2, and 0.5 mA. for the last for pt. D_2).

Comparable rises in the intensity of the effective current at certain frequencies were noted. The first comparable rise in the intensity of the thresholds of these 2 points were

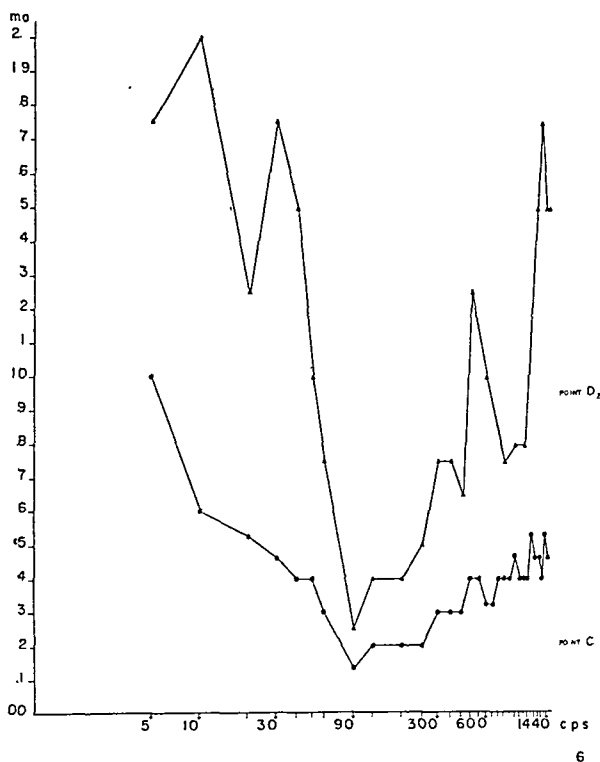


FIG. 6. The liminal intensities of the sinusoidal currents (5 to 1440 c.p.s.) giving movement were plotted in semilogarithmic coordinates. The upper curve (labeled point D_2) was found on M 's precentral gyrus; the lower curve (labelled point C), on D 's precentral gyrus.

found at 300 c.p.s. (0.33 mA. for pt. C and 0.75 mA. for pt. D_2) and another at 480 c.p.s. (0.4 mA. pt. C: 1.25 mA., pt. D_2). Both fell with stimulation with 600 c.p.s. (0.33 mA., pt. C: 1.00 mA., pt. D_2), and were higher again together with 840 c.p.s. (0.4 mA. pt. C: 1.25 mA., pt. D_2). They rose together with 1140 c.p.s. (0.46 mA., pt. C: 1.5 mA., pt. D_2) and with 1260 c.p.s. (0.46 mA., pt. C: 1.75 mA., pt. D_2). With 1380 c.p.s. the threshold for the movement from point C was higher (i.e., 0.53 mA.) and for point D_2 lower (i.e., 1.5 mA.), and with 1440 c.p.s. the threshold was lower again (0.46 mA.) for point C, but for point D_2 , similar (1.5 mA.).

The intensity of the stimulating frequency which produced the movement increased consistently with the frequencies less than 90 c.p.s. for point C on D 's cortex, (0.33 mA., 60 c.p.s.; 0.4 mA., 50 and 40 c.p.s.; 0.46 mA., 30 c.p.s.; 0.50 mA., 20 c.p.s.; 0.6 mA., 10 c.p.s.; and 1.0 mA., 5 c.p.s.) but for point D_2 on the cortex of M , the effective intensity for

30 c p s was greater than that of 20 c p s, and the 10 c p s, greater than 5 c p s (0.75 mA, 60 c p s, 1.0 mA, 50 c p s, 1.5 mA, 40 c p s, 1.75 mA, 30 c p s, 1.25 mA, 20 c p s, 2.00 mA, 10 c p s, 1.75 mA, 5 c p s)

Stimulation of point C produced extension of the ankle with each cycle used with the exception of 360 c p s. Stimulation of point D₂ produced adduction of thumb with each cycle used with the exception of 10, 360, and 1260 c p s. Strangely enough the stimulation with the 360 c p s on each cortex produced deviation—point C gave abduction of all toes and extension of the hallux and point D₂ yielded flexion of all fingers and flexion of the elbow. Both 10 and 1260 c p s on point D₂ produced a deviation—flexion of the thumb rather than adduction. The liminal stimulus for each cycle used did not always give the simple movement of extension of ankle (point C) or of adduction of the thumb (point D₂). Co innervations were elicited. Those accompanying extension of the ankle were most frequently another movement of the ankle (eversion, 8 X, inversion, 6 X) and those with adduction of the thumb were movements of the forearm (supination 9 X, pronation 1 X) or of the wrist (radial, 4 X, or ulnar 1 X flexion). Extension of the toes was observed five times and flexion of the toes once as co innervation with extension of the ankle (point C) and five times flexion of the fingers and twice extension and once flexion of the index finger were seen as co innervations with adduction of the thumb (point D₂).

Point C was stimulated with a supraliminal strength (0.66 mA) kept constant for each of the 31 cycles per sec used. For 21 of these frequencies a similar movement was obtained—that of extension of the ankle, flexion of the lateral 4 toes and adduction of the hallux—which may be designated as movement "A". Three different additive movements were obtained with the use of the 10 remaining cycles, flexion of the hallux (120, 180, 780, 840 c p s), eversion at the ankle (360, 420, 480, 540, c p s), and inversion (600 and 720 c p s). These three additive movements, obtained with supraliminal stimuli, contained all the varieties of muscle contraction elicited with the liminal stimuli for some of these frequencies with two exceptions (i) extension of the toes, and (ii) abduction of the hallux. Therefore, if the repetitive stimulation of this point be considered as a whole, reversal of a part of the movement obtained with the liminal stimulus was elicited with the supraliminal stimulus, for the extension of the toes became flexion with the following frequencies—660, 1200, 1260, 1320, 1380, and 1440 c p s. With 660 c p s the abduction of the hallux obtained with the liminal stimulus became adduction with the supraliminal stimulus. The augmentations of movement "A" with either inversion or eversion at the ankle yielded by the supraliminal stimulation with all but one of the above cycles (360) were facilitations of the movements obtained by the liminal stimuli delivered by each of these frequencies. With 360 c p s the supraliminal stimulus yielded a deviation of the movement produced by the liminal stimulus. Two of these frequencies refused to produce movement "A" with the strength of current which was sufficient to produce that movement with the remaining frequencies, for 1.0 mA of the 10 c p s and 1.3 mA of the 5 c p s were required.

Besides these characteristics of the response of a given cortical point to liminal and supraliminal stimuli of different frequencies, there was a measurable delay between the time when the given point was touched with the stigmatic electrode and the initiation of movement. For the supraliminal stimuli delivered to point C, this measurable delay (stop watch, graded in 0.01") was found with certain of the higher cycles (1200 c p s, 1.4', 1260 c p s, 0.9", 1380 c p s, 1.0') and with each of the 7 lowest (60 to 5) frequencies used. The longest delays occurred with 50 (1.7"), 20 (1.9'), 10 (1.1") and with 5 (2.0') c p s. For the liminal stimuli given the two points, D₂ and C, the movements elicited with certain of the higher frequencies (1140 to 1440 c p s) were less delayed in their initiation (range 0.5" to 1.2", for pt C and 1.2' to 2' for pt D₂) than were those with the lower frequencies (5 to 60 c p s, range 1.0" to 2.0' for pt C, and 1.5" to 4.8" for pt D₂).

These delays were those recorded for the liminal intensity of the stimulating frequencies, capable of reproducing the movement initially elicited by a threshold stimulus of the 60 c p s current applied to the chosen cortical point. In the search for the thresholds several intensities and their corresponding periods of delay were recorded with certain frequencies. For example, point D₂ (M's cortex) yielded some movement when stimulated with the following frequencies at the following intensities of current and their respective duration of applied stimulation as outlined in Table 1. Similar data were also obtained when point C (D's cortex) was stimulated with certain cycles (Table 2). Although the above data are obviously incomplete, they are sufficient to indicate that delay may be

a function not of the frequency only but also of the intensity of a given stimulating double vibration. Further, the height of the contraction elicited was reached more slowly when the chosen point was stimulated with the lower cycles (5 to 30 c.p.s.) than with any of the higher ones.

Table 1. Point D_2 (M 's cortex)

c.p.s.	I in mA.	Duration in sec.
5	1.5	5.1
	1.75	4.6
10	1.75	5.2
	2.0	3.9
20	1.0	12.9
	1.25	3.3
30	1.5	11.6
	1.75	3.0
60	0.5	8.0
	0.65	1.5
	0.75	0.0
90	0.25	3.5
	0.30	1.6
	0.40	0.0
120	0.40	1.6
	0.50	1.6
	0.75	1.0
300	0.7	2.7
	0.75	2.6
420	0.65	3.0
	0.75	1.0
600	1.0	3.8
	1.25	0.0
960	1.25	6.0
	1.5	3.9

Table 2. Point C (D 's cortex)

c.p.s.	I in mA.	Duration in sec.
5	0.6	2.0
	0.66	2.0
	1.0	1.8
10	0.6	1.9
	0.66	1.0
	1.0	1.1
20	0.53	2.0
	0.66	1.9
30	0.46	1.3
	0.66	0.9
40	0.4	1.5
	0.66	0.9
50	0.4	1.8
	0.66	1.7
60	0.2	2.1
	0.26	1.8
	0.36	2.0
	0.4	1.6
240	0.66	0.5
300	0.2	1.8
	0.46	0.5
300	0.46	1.1
	0.66	0.3
480	0.36	3.0
	0.4	1.0
1200	0.4	0.9
	0.46	0.5
	0.66	1.4

The quality of the movement made by these muscles appeared to be a function of the cycle used. The movements elicited by stimulation either of point D_2 (M 's cortex) or of point C (D 's cortex) with frequencies of 20 or 30 c.p.s. were slow, deliberate and smooth; with 10 c.p.s., slow and jerky; and with 5 c.p.s., slow, tremulous and jerky but not decomposed into component parts. The movements elicited by frequencies greater than 90 c.p.s. were neither abrupt nor deliberate, nor were they tremulous. In the case of point D_2 on the cortex of M , jerky incompleted movements were elicited with the 3 highest cycles used (1260, 1380 and 1440); but in that of point C the movements were jerkily executed only at the highest frequency, 1440 c.p.s.

DISCUSSION

Although analysis of movements elicited by electrical stimulation of the precentral gyrus can furnish only a first approximation to an understanding of the functional contribution of that cortex to the motor performance of the animal in question, nevertheless comparison of results of such studies in different animals has demonstrated that the resulting movements bear a direct relation to those which characterize the animal so stimulated (Hines and Boynton, 1940). This is particularly true if the current used be sinusoidal and the frequency, optimum or within its neighborhood. For the isolated movements and the co-innervations which were elicited could be correlated directly with those observed in the living animal at the time of the cortical exploration. Co-innervations include use patterns and their supporting movements, behavior patterns and patterns of progression or of posture. The first two can be elicited under deep ether anaesthesia. The last two can never be elicited in their entirety except under very light ether anaesthesia. The results of the stimulation of these three chimpanzee cortices can be analyzed in a similar way, except that throughout these explorations the anaesthesia was so deep that progression patterns were obtained only in fragments.

Contractions of isolated muscles were elicited. Certain of these isolated movements were obtained from each one of these cortices. The points from which these isolated movements were produced covered large cortical areas and suggested that cortico-fugal fibers are more numerous to certain motor nuclei within the segmental apparatus than to others. Thus the cortico-fugal fibers to the motor nuclei which supply the adductors of the first digits and the flexors of the lateral four could be considered as more numerous than those which supply the abductors of the first and the extensors of the remaining digits, while the projection fibers for such movements as opposition of the thumb, flexion or extension of the first digits, and flexion of the 2nd and of the 5th fingers would be relatively few. Similarly, isolated contractions of muscles were elicited by electrical stimulation of the face area, such as contraction of separate groups of fibers of the intrinsic muscles of the tongue and of those of the *M. platysma* or of the contralateral *M. glossopharyngeus*. It is evident then that it is possible to innervate separate motor nuclei within the spinal cord (pyramidal fibers apparently end among their component cells, Leyton and Sherrington, 1917) as well as functionally discrete portions of the nuclei of the *N. facialis*, or the *N. hypoglossus*, or the nucleus ambiguus of the *N. vagus*.

Certain combinations of the compound movements often recurred. Many of these co-innervations represented activation of the dorso-lateral or the ventro-lateral groups of nuclei within the spinal cord, innervating respectively the flexor sheet or the extensor sheet of muscles. Here, the cortical stimulation might be considered as activating spinal cord mechanisms rather than discrete nuclei and therefore as passing over the pyramidal terminals at the base of the dorsal horn (monkey, Schafer, 1884, 1899; monkey

and cat, Hoff, 1932). Thus, the occurrence of supination of the forearm with flexion of the elbow, and of pronation with extension of that joint, of protraction of the thigh with flexion of the knee, of adduction of the 1st digits with flexion of the lateral 4 or of abduction of the 1st with extension of the remaining 4, fell into this grouping.

Frequency of elicitation of certain movements as co-innervations and of others as isolated may be correlated with patterns of progression or of posture. When included as co-innervations with other movements, protraction of the thigh, flexion of the knee, retraction and abduction of the upper arm were more dominant than their respective antagonists. Certain other movements were always more frequently elicited than their antagonists, namely, retraction of the upper arm, external rotation of the thigh, pronation of the forearm and extension and inversion of the ankle. And additive extension of the digits was more frequent than additive flexion of the digits. The chimpanzee cannot extend its fingers completely, for the flexors are too short. In that ape's quadrupedal progression the forelimb is supported upon the surface of the middle phalanx of the 2nd and the 3rd fingers; the wrist is extended; the forearm is pronated; the upper arm is more abducted than adducted, more retracted than protracted; the thigh is more protracted than retracted; the knee is only partially extended; the ankle is extended (*i.e.*, dorsi-flexed) and slightly inverted; the toes are loosely extended and the great toes abducted. In bipedal progression these characteristics of muscle use are emphasized. There is little fixation of the trunk; the thighs are abducted and slightly externally rotated; the knees are kept a little flexed; the ankles remain slightly inverted, so that progression proceeds as a side to side movement. With each protraction of the thigh the trunk flexes slightly. In brachiation partial extension of the fingers follows the sequence of movements initiated in more proximal muscles, retraction or abduction of the upper arm, and flexion of the elbow. In sitting the thighs are greatly abducted and externally rotated, the knees flexed, the ankle semi-extended and inverted; the upper arm is generally kept in semi-abduction, the elbow flexed and the forearm is in supination.

Besides these movements, co-innervations of flexor and extensor muscles were observed. These types of compound movements may be allocated to patterns of cortical selection. The use patterns of the distal muscles of the hand and foot require variegated co-innervations of supporting movements. Thus supination and pronation occurred with either extension or flexion of the wrist, or with either extension or flexion of the fingers; and flexion of the knee either with extension or flexion of the ankle. The use patterns themselves included those cooperative movements of the digits such as serial flexions, opposition of the 1st to one or more of the remaining in flexion and abduction of the 1st with extension of the 2nd.

A comparison of dominant isolated and compound movements with those outlined by Foerster for man (1936, pp. 48-49) clarifies the initial generalization, that the results of the stimulation of the precentral gyrus

bear a direct relation to the movements which characterize the normal usage of the animal in question. The chimpanzee cortex resembles that of man in the dominance of flexion of the digits and adduction of the first digit, of extension at the ankle and of flexion at the elbow, and of isolated retraction of the thigh and isolated extension of the knee; and differs from that of man first, in the dominance both of certain co-innervations (those of protraction at the thigh and those of flexion at the knee) and of retraction and abduction of the upper arm and pronation of the forearm, and second, in the infrequency of elicitation of opposition of the thumb and of isolated movements of individual fingers. No isolated movements of either the 3rd or the 4th fingers were obtained. They were also absent from Sherrington's series of 42 chimpanzees (total count of animals reported as used, 1901-1917)! Conversely, the results of electrical stimulation of the chimpanzee face area elicited isolated movements not as yet reported for man. Such movements were of the posterior, the middle or the anterior fibers of the *M. longitudinalis superior* of the tongue, the vertical fibers of the tongue, the contralateral *M. glossopalatinus* and parts of the *M. platysma*, and of the *M. genioglossus* (posterior part only).

The topographical projection of somatic musculature upon the precen-tral gyrus of these three chimpanzees (Fig. 4 and 5) varied in a few particu-lars from that given by *Sherrington* (1901). The areas of the thumb and upper lip v and Sherrington inter-polated neck muscles between these two regions. However, examination of the maps given by *Leyton* and *Sherrington* (1917) show that movements of muscles of the neck were not invariably elicited. Stimulation of the face area of the foetal *Macaca mulatta* yielded movements of the muscles of the neck (*M. sterno-cleido-mastoideus*, for example) (*Hines* and *Boynton*, 1940) and of others attached to the shoulder girdle at a time in development before any movement of facial muscles could be produced, whereas later (older) such movements were obtained only occasionally when the ether was very light. Such might account for their sporadic occurrence in *Leyton* and *Sherrington's* experiments. That *Krause* (1912, p. 291) did not obtain them in man while *Foerster* (1936) and *Penfield* and *Boldrey* (1937) did, might be correlated with the conditions of their respective stimulations. *Krause's* pa-tients were under a general anaesthesia.

Unlike *Leyton* and *Sherrington* the writers found different loci for an-tagonist movements of certain joints. Flexion of the fingers was ventral or anterior to extension; extension of the toes, ventral to flexion of the toes. Supination and pronation were generally placed dorsal to flexion or extension of the wrist. Flexion of the ankle (in *D*) was found ventral and caudal to extension of the ankle. And in this animal flexion of the hallux was ventral to the greater number of points giving adduction of that digit. These find-ings resemble in part *Forester's* for man. *Penfield* and *Boldrey* (1937) and *Krause* (1912) have found flexion and extension of the fingers separated in individual cortices.

The broad outlines of the projection of the remaining soma upon the precentral gyrus resembled that of Grünbaum and Sherrington. Movements of muscles of the trunk were found between those of the scapula below and the thigh above. However, in each of these animals, stimulation of a single point in this region produced not only movements of the chest wall but also changes in respiration—a finding unreported by other investigators. In this same general region not only were bilateral movements of the abdominal musculature obtained but also those of the thigh and knee. These latter movements resemble those obtained by Foerster with strong faradic stimulation. Similar movements were elicited in the young monkey (Hines and Boynton, 1940), and in the adult monkey after bilateral section of the medullary pyramids (Tower and Hines, 1935) from the anterior border of area 4. Here the excitation travelled by extrapyramidal projection fibers. Foerster, however, believed the movements he obtained in man travelled by ipsilateral pyramidal fibers.

Contraction of the *Mm. constrictor ani et levator ani* were found most ventral and anterior on the paracentral lobule. Movements of the thigh, the knee, the ankle and the toes were located there also in a manner which resembled more closely the findings of Foerster for man than those of Grünbaum and Sherrington for the chimpanzee.

Leyton and Sherrington reported visible movements of the vocal cords but no vocalization; Walker and Green (1938), vocalization "in only one experiment" by stimulation of the face area of the macaque. The localization of the two points from which a vocalization (V, Fig. 1) and a light whistle (W, Fig. 1) were elicited on the *M*'s precentral gyrus resembled those found for man by Penfield and Boldrey (1937) and by Penfield (1937). The point (see S in Fig. 1) from which salivation was elicited was located on the anterior crest of the central fissure. Salivation was produced by Walker and Green by stimulation of area 4 and area 6 (unspecified primate) and by Penfield and Boldrey (man) by that of the lower part of the postcentral gyrus (2 instances).

A few points on the postcentral gyrus responded without previous facilitation, therefore resembling the findings of Penfield and Boldrey (1937) for man rather than those of Sherrington for the chimpanzee. The writers have also been able to elicit movements which were neither synergic nor adverse not only from the postcentral gyrus (areas 3, 1, 2) but also from the parietal lobule of the macaque (adolescent and infant) without initial facilitation. This result may be correlated with the difference in the type of current used.

The stimulation of a few points within area 6, located on the caudal limb of the superior, middle and inferior frontal gyri elicited no adverse movements but rather movements which resembled Foerster's (1936, man) so-called synergies in contralateral musculature. These movements were protraction of the thigh and flexion at the knee with a single instance of additive extension of the ankle and flexion of the toes, retraction at the shoulder and flexion of the elbow, external rotation of the shoulder or of the upper

arm. While protraction of the thigh and flexion of the knee or the retraction of the shoulder and flexion of the elbow are partial synergies, they are also components in progression, to which extension of the ankle and the flexion of the toes are added. Similar movements have been observed by the writers to follow stimulation of homologous areas in young monkeys under light anaesthesia.

In the general exploration of the precentral gyrus with the 60 c.p.s. sine wave current, the amperage of the liminal stimuli was found to be lower in the face area than in either the arm or leg areas in all these animals. And the cytoarchitecture of the exposed surface of the face area is largely 6 (Campbell, 1903; Brodmann, 1912; Bucy, 1935; Walker and Green, 1938). Therefore unless the anterior limit of area 4 in these three precentral gyri be different from that outlined by these investigators, the lowest threshold was found in area 6.

On the other hand points located on the rostral crest of the inferior precentral fissure (area 6, Brodmann, 1912) had higher thresholds than any point located on the precentral gyrus itself. Again points on the anterior border of the leg and arm areas (no fissure present) had higher thresholds than those placed more rostrally. This sudden rise in threshold during the stimulation of a row of points probably indicated that the anterior boundary of the area gigantopyramidalis was passed; for such a finding, later checked by histological studies, had repeatedly been found to be the case in previous studies (Boynton and Hines, 1933; Hines, 1937; Hines and Boynton, 1940) of this area in the brain of the *Macaca mulatta*.

All but a few low threshold points on the arm area yielded either isolated or primary movements of the fingers. No such similar distribution of low thresholds was found in the leg region, for movements of the thigh as well as the toes were found among them. Moreover, it was (Hines and Boynton, 1940) found that the lowest thresholds of points on the arm and leg areas of the young monkey gave, respectively, movements of the fingers and of the toes, when stimulated with the 60 cycle sine wave current.

The definite minimum in the curves produced by plotting in semi-logarithmic coordinates the liminal peak amperage of the range of frequencies used to stimulate single points on the surface of the precentral gyrus occurred at 90 c.p.s. On either side of this optimum frequency (Coppée, 1936) the curve rose. Nevertheless, the range of lowest threshold frequencies was similar for the two curves presented in Fig. 6 (50 to 420 c.p.s.). Similar findings were reported as characteristic of homologous points similarly stimulated on the adult or year old macaque cortex (Hines and Boynton, 1940). Cooper and Denny-Brown (1927) recorded optically the responses of the *M. brachialis anticus* (*Macaca mulatta*) to cortical stimulation with a series of break shocks (35 to 90 p.s.) and with a rotating commutator (20 to 300 p.s.). They found that flexor muscles follow cortical stimulation up to 180 to 200 p.s. At higher rates the response became irregular and half rates were recorded. At lower rates of stimulation secondary waves were intro-

duced irregularly to make a total which varied from 180 to 200 ps. Rates lower than 8 p.s. rarely produced any response. But with 90 p.s. one primary response followed each stimulus and for each primary there was a secondary response establishing a regular double rhythm. The greatest regularity of response was to the rate of 90 p.s.; that is, the optimum frequency which we found for both the adult chimpanzee and for the adult macaque. It is just possible that this frequency is peculiar to these primates; for similar experiments on 2 other mammals the optimum frequency was different (Boynnton and Hines, 1933; Straus and Hines, 1934).

Further, we noted that the delay between the time at which the electrode was applied to the cortical surface and the appearance of the contraction of the muscle was greater with the lower frequencies (60 to 5) than with the higher ones (660 to 1440). Cooper and Denny-Brown called this period of delay, the "summation period," and were able to measure it accurately. This "summation period" at times varied as much as 100 per cent with similar rates, in spite of care to avoid facilitation. Nevertheless as the rate per second decreased, the average "summation period" increased. There is also in our explorations, in spite of the unaccountable variables in such experiments as these, a relationship not only between the "summation period" and the frequency but also between the "summation period" and the strength of the stimulating current. For in general, the lower the intensity of current, the longer the "summation period" required to elicit movement; and below the optimum frequency the lower the frequency the longer the "summation period." With the highest frequencies used (1200 c.p.s. and above) the "summation period" increased. The rate of accumulation of excitation within the motor neurones then appears to be a function of the frequency as well as of the intensity of the stimulating current. But all frequencies used in these explorations produced movements. And the movements produced by stimulation of this cortical point with all of these frequencies showed no greater range of variability than those elicited by different intensities of a single frequency. What did vary was the quality of the movements elicited. The highest frequencies elicited jerky, incomplete movements, those of 30, 20 and 10 c.p.s. produced slow and deliberate movements, while to that of 5 c.p.s., the responding movements were not only slow but also tremulous. Apparently the central nervous system of the chimpanzee was able to accumulate all but the lowest frequency of excitation used; for tremors in somatic musculature are produced by rates of stimulation of peripheral nerves of 15 or less p.s. (Bronk, 1936). But the movements which resembled those seen in the unanaesthetized animal were elicited by the frequencies in the neighborhood of the optimum.

SUMMARY

The precentral gyri of three adult chimpanzees were explored with the 60 c.p.s. sine wave current. The movements elicited were interpreted as activation (i) of discrete motor nuclei of the cranial and spinal nerves and

of parts of these nuclei; (ii) of groups of motor nuclei within the spinal cord which innervate extensor or flexor sheets of skeletal muscles; and (iii) of brain stem integration systems. Certain of the co-innervations suggest cortical selection of nuclear groups, such that use patterns were elicited.

The most frequently elicited movements of skeletal muscles were those of the digits, flexion of the lateral four and adduction of the first. Isolated movements of 2nd or 5th fingers were rare; those of the 3rd and 4th fingers did not occur. Different loci for antagonistic movements at the joints were obtained.

Vocalization, a light whistle, and salivation were produced in one chimpanzee. The postcentral gyrus responded without previous facilitation.

The liminal strength of the stimulating frequency for area 4 (arm and leg) was higher (0.22 to 2.0 mA.) than for the face area (largely area 6) (0.2 to 1.5 mA.).

A single point on two of these precentral gyri was stimulated with a range of double vibrations (5 to 1440 c.p.s.) of the sinusoidal current. 90 c.p.s. was the optimum frequency for each of these points. The frequencies eliciting movements which in their timing, sequence, and smoothness resembled characteristic of the unanaesthetized animal were those in the neighborhood of the optimum.

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SENSORY CORTEX OF CHIMPANZEE*

PERCIVAL BAILEY,† J. G. DUSSER DE BARENNE, HUGH W. GAROL;
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(Received for publication June 26, 1940)

INTRODUCTION

Errata for J. Neurophysiol. vol. 3, (no. 4), July, 1940

Errata for *J. Neurophysiol.* vol. 3, (no. 4), July, 1940

p 288 19a. Experimental results on sensory localisations in the cerebral cortex. *Quart. J. exp. Physiol.*, 1916, 9: 355-390

... the preliminary paper, namely local
... the cerebral cortex in the fully anesthetized animal and
investigation of the distribution of the "strychnine spikes" appearing in the

Errata for J. Neurophysiol. vol. 3, (no. 4), July, 1940

p. 291 119a. The knee-jerk following facilitating and extinguishing stimulation of related cortical foci. *Amer. J. Physiol.*, 1939, 126: 570 (with W. S. McCulloch)

animal.

For more than thirty years the use of local strychninization of the central nervous system (CNS) has proved itself an almost ideal method for the study of the location and functional organization of sensory systems in the CNS. In 1909-1913 the senior author (5) applied this method to the spinal cord. In 1915 he (6) found that local strychninization *within* a certain region of the cerebral cortex of the cat, after recovery of the animal from the operative anesthesia, results in transient—for about 30 minutes—typical symptoms of sensory excitation: paraesthesiae and hypersensitivity of the skin and deeper structures. Strychninization *without* this region did not result in sensory disturbances. In 1923 the same author (7) using the same methods, namely local strychninization in conjunction with "clinical" observations of the animal, delimited the sensory cortex of the macaque monkey. The obvious conclusion from these two series of experiments is that the region thus delimited subserves somatic sensory functions, is the "sensory" cortex.

After the "sensory" nuclei of the thalamus opticus of the cat had been diagnosed by the same physiological methods (14), Dusser de Barenne and McCulloch (10) showed that local strychninization of the sensory cortex, as ascertained by the first combination of methods, results—even in the fully anesthetized animal—in activation of these very same thalamic nuclei:

* Aided by a grant from the John and Mary R. Markle Foundation

† On leave of absence from the Department of Neurology and Neurosurgery, University of Illinois

‡ Honorary Research Fellow, Yale University School of Medicine.

local strychninization of the various subdivisions of the "sensory" cortex results in the appearance of large and rapid voltage fluctuations—"strychnine spikes"—in the electrograms of the corresponding "sensory" nuclei of the thalamus.

Finally Dusser de Barenne and McCulloch (9) and these authors with Ogawa (13) studied the distribution of the strychnine spikes in the electrograms of the cerebral cortex following its local strychninization in the fully anesthetized monkey. Again the same region was as delimited with the original combination of methods. Local strychninization *within* this region resulted in widespread strychnine spikes with a typical distribution following strychninization of each constituent area; strychninization *without* this region never resulted in the appearance of strychnine spikes in the electrograms taken within the region. Furthermore, the distribution of the strychnine spikes is specific for each constituent area of this region. The obvious conclusion from these various pieces of experimental evidence is that the distribution of the strychnine spikes in the cerebral cortex following its local strychninization permits one to determine the location, extent and functional organization of the "sensory" cortex even in the fully anesthetized animal.

"Clinical" observation of as strong an animal as the anthropoid ape after local strychninization of its cerebral cortex would be too dangerous for the experimenter. We have, therefore, for the study of the chimpanzee's sensory cortex, employed the combination of methods which can be used in the fully anesthetized animal. Again this combination of methods has revealed on the surface of the hemisphere a very large region, the location, extent and functional organization of which is comparable to that of the sensory cortex of the macaque monkey. The obvious inference again is *that the region so disclosed is the sensory cortex of the chimpanzee*.

On all hemispheres of this new series, except one, extensive electrical stimulations of the cortex were performed with special stimulators (see the section on methods) allowing the use of various pulseforms. The relevant findings of these stimulation experiments and their correlation with the findings in this paper will be published in a separate paper.

METHODS

These experiments were performed on 5 immature, 2.5 to 3.5 years old, chimpanzees (*Pan satyrus*), fully anesthetized with Dial* (0.35–0.45 cc. per kg. body weight, half of the doses given intraperitoneally, half intramuscularly). When the animal was fully under narcosis the head was clamped in a special head holder, and the body placed on an inclined board so that the head was lower than the hindquarters of the animal; this was done to maintain proper cerebral circulation.

Each investigation on one animal lasted from 3 to 3.5 days without interruption. It was, therefore, necessary to work in day- and nightshifts, usually from 12:00 noon until 12:00 midnight and from 12:00 a.m. until 12:00 p.m.† During the first 1.5 to 2 days the

* We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

† We wish to extend our thanks to Messrs. Willard B. Chamberlain, Craig W. Goodwin, John M. Hamilton and Arthur A. Ward for their unremittingly enthusiastic assistance in these long and weary experiments.

sensory cortex of one hemisphere was investigated, the rest of the time was devoted to the exploration of the second hemisphere

After exposure of the larger part of the convexity of one hemisphere several photographs of the exposed region were taken from different angles to get as little perspective distortion as possible in the final composite drawing of the hemisphere. Lifesize prints were made to record accurately the location and extent of the several local strychninizations and of the electrically excitable "motor" cortex

The investigation of each hemisphere was begun with a careful exploration of the "motor" cortex. For this a specially constructed stimulator designed and built by Mr Craig W. Goodwin, the electronic engineer of this laboratory, was used. * This apparatus allows independent variation of the rising and falling phases of each individual pulse and the frequency of the pulses per second, the pattern frequency of the stimulation, within a wide range, and the number of pulses per stimulation. For several years Dusser de Barenne and McCulloch had known (from unpublished experiments) that with long pulses—of several sigmas' duration—the voltage required to stimulate the "motor" cortex is much lower than with short pulses and that especially the duration of the descending phase seems significant, under moderate Dial anesthesia a descending phase of from 8 to 12σ duration is optimum. Where necessary, due caution was taken in regard to the disturbing influence of facilitation and extinction (8). In other instances facilitation was used as a useful factor in determining the most frontal boundary of the "motor" cortex. An important feature of the stimulatory exploration was also the delimitation of the boundaries between the leg, arm and face subdivisions of the "motor" cortex. For further details see the next paper on the "motor" cortex of the chimpanzee.

After the careful exploration and mapping of the "motor" cortex the experiments with local strychninization were begun. Because of the tremendous functional complexity of the chimpanzee's cortex it was decided to concentrate our efforts in this group of animals mostly on the arm subdivision, in the last chimpanzee attention was focussed predominantly on the leg subdivision. Six columns of 6 stigmatic Ag-AgCl₂ electrodes were placed on the exposed portion of the cortex, the uppermost of each column high up in the leg subdivision, dorsal to the boundary between the leg and arm subdivisions, the 5 others of each column were spread vertically over the dorso-ventral extent of the arm subdivision. In several of the experiments the most ventral electrode of each column was placed in the face subdivision. Thus, although in these experiments most information was obtained about the arm region of the sensory cortex, a good deal of evidence about the leg and face regions and the functional boundaries between the three major subdivisions of the sensory cortex was also acquired. On the precentral cortex the results of the electrical explorations gave useful information for the placement of the electrodes, on nearly all of the postcentral cortex this guiding factor was not available and we relied on experience with the functional boundaries obtained in the chimpanzees of the preliminary paper.

The wires from all 36 electrodes were connected to a 6 pole—6 throw switch and from this to the 5 amplifier sets of 5 Grass inkwriter oscillographs in such a way that in one position of the switch all 6 electrodes of one column were connected to the oscillographs. Thus, by turning the switch, each column of electrodes on the cortex could quickly be connected with the amplifiers and oscillographs. If we call the 6 electrodes in each column from above downwards, a, b, c, d, e and f and the 5 oscillograph channels A, B, C, D and E, the hook up was such that electrodes a and b were connected to channel A, electrodes b and c to channel B, electrodes c and d to channel C, electrodes d and e to channel D, electrodes e and f to channel E. A change or disturbance of the electrical activity in the cortex under or near electrode a will affect only channel A,

and a disturbance under or near electrode f and a change in the ECG of channel E by itself a disturbance under electrode f. Thus each change in the ECGs of any channel or group of channels reflects a change in the activity of the cortex under or near a particular electrode or group of electrodes. Thus, in turning the 6 pole—6 throw switch one can investigate in quick succession the ECGs of the cortex under or near the 6 electrodes of each of the 6 columns.

The taking of the ECGs before and after each strychninization constitutes one experiment, whose actual course usually was as follows: (i) the taking as "control runs" for 1 min of the "normal" ECGs for each of the 6 columns of electrodes, (ii) the local strychninization.

* Mr Goodwin will describe this stimulator separately in the near future.

ninization of the cortex by applying a small piece of filterpaper of appropriate size and shape soaked in a 3 per cent solution of strychnine sulfate colored with toluidine blue; (iii) the taking of half-minute runs of ECGs for each of the 6 columns of electrodes in succession until any change, if such resulted from the strychninization, had passed off. Five to ten minutes after its application the filterpaper was removed and any fluid at the site of strychninization carefully blotted. Usually after 25 to 45 minutes the effects of such a strychninization had passed off, depending upon the depth of narcosis, the circulation, etc.

In our work on the macaque's cortex truly local strychninizations were always performed, *i.e.*, strychnine-filter papers of a few square millimeters applied. In the chimpanzee the effects of such local strychninizations are very much more restricted than in the

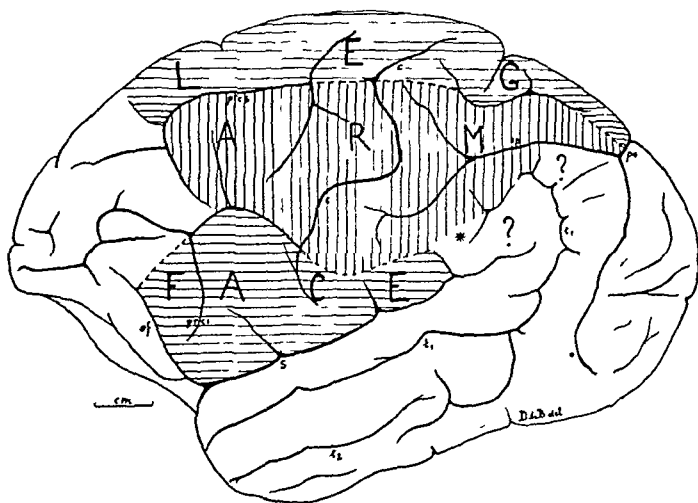


FIG. 1. Sensory cortex of the chimpanzee. This figure represents as accurately as is possible in one plane the outer surface of one hemisphere of chimpanzee No. 4. The precentral boundary between the face- and arm-subdivisions lies in this hemisphere unusually low, but was verified both by electrical stimulation with motor response and with the strychnine method. It is also unusual that this boundary is marked by a definite sulcus across the precentral gyrus. The figure schematizes the results of over 200 observations on this one brain.

macaque; therefore, the strychnine filterpapers used in the chimpanzee experiments were taken larger, 3×5 or 2×10 millimeters.

Thus, in general, during each experiment from 8 to 14 series of ECGs from the 6 columns of electrodes were taken. Usually between 15 to 25 strychninizations were performed on one hemisphere, *i.e.*, 30 to 50 experiments performed on the brain of each chimpanzee.

At the end of such an investigation, lasting without interruption for 3 to 4 days, the brain was injected through the carotids with 15 per cent neutral formalin, then removed from the skull and placed in formalin. After 12 to 24 hours the brain was weighed and carefully measured. After a few days the soft membranes were peeled off and the brain photographed before and after removal of the brainstem and cerebellum and after splitting in the midplane. Finally all the strychninizations were carefully mapped on life size photographs of the hemispheres.

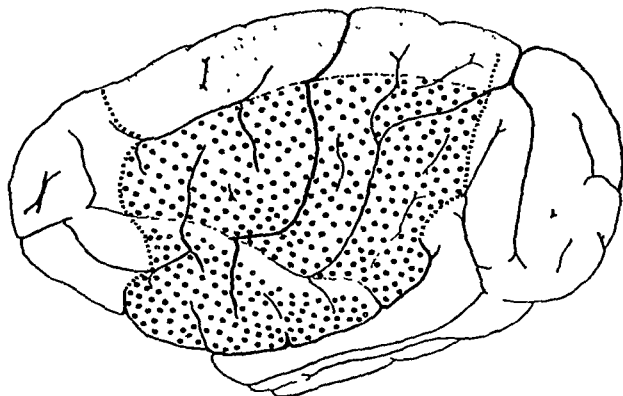
Then the analysis of the several thousand feet of record was begun and the change of the ECG under each electrode in each experiment marked on drawings (twice life size) of each hemisphere ("firing," suppression, "spindles," increase of activity, no change). Thus the final plotting of the results of each experiment on the cortex of one hemisphere is based on the study of circa 400 ECGs (each comprising 8 or more records) and the final composite diagram, on that of well over 100,000 records.

RESULTS

Location, extent and subdivision of sensory cortex

The experiments of this new series have essentially confirmed the location and extent of the sensory cortex of the chimpanzee as given in the preliminary report and as reproduced in Fig. 1. They have permitted us to answer a few of the questions left in this diagram. Those small areas marked there with a ? do not belong, as we can definitely state now, to the sensory cortex.

We are also in a position now to state that the small triangular area



SENSORY CORTEX OF CHIMPANZEE

FIG 2 A composite drawing showing the extent and location of the sensory cortex and its subdivisions for leg (. . .), arm (. . .) and face (. . .). The regions for trunk (between arm and leg) and for neck (between arm and face) are left blank
 X X X = anterior and posterior margins of the sensory cortex, — . . — = boundaries of arm-subdivision

marked with an * in the first diagram belongs to the arm-subdivision. The posterior border of the sensory leg cortex did not extend in the last 5 animals all the way to the fissura parieto-occipitalis externa (ope), as was the case in the fourth animal of the first series, apparently individual variations are not infrequent.

In Fig. 2 is given an "average" (see discussion) aspect of the convexity of the chimpanzee's brain; in it are indicated also the location, extent and subdivision of the sensory cortex as delimited in these new experiments. It will be seen that this cortex occupies a large portion of the hemisphere on its outer surface both before and behind the fissura centralis and comprises three major subdivisions: the leg-, arm- and face-subdivisions. Between the

leg- and arm-subdivisions lies a narrow strip of cortex in which presumably the trunk is represented sensorially. This statement is based on (i.) the existence in the precentral cortex of an intermediate "motor" trunk region and (ii.) the occurrence of small or questionable strychnine spikes within both the pre- and postcentral portion of this intermediate region from strychninizations definitely in the leg- or arm-subdivision. Between the arm- and face-subdivisions lies a narrow strip of cortex in which presumably the neck is

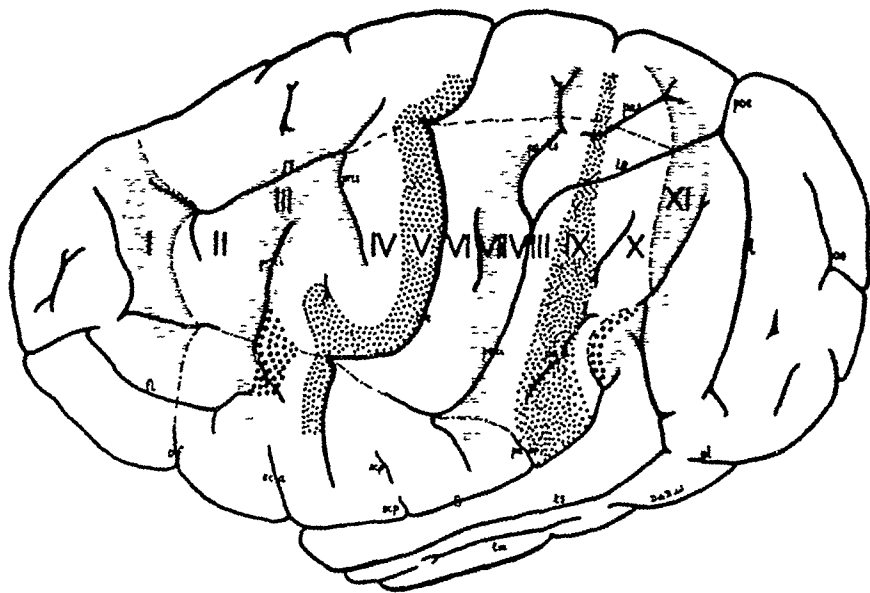


FIG. 3. The extent, location and functional subdivisions of the sensory arm cortex into physiologically distinguishable bands, Nos. II-X, and of the immediately adjacent bands, Nos. I and XI, indicated on a composite drawing representing the arm area in the center of the field. The bands giving suppression, Nos. I, III, VII and XI, are marked thus: ≡≡≡. Small ••• indicate bands V and IX. Large ••• mark the "dud" areas. Areas between trunk and arm and between arm and neck are marked ---.

represented sensorially. The reasons for this statement are the same as for the trunk region, *mutatis mutandis*.

While we refer to this region as the "neck" region it should be remembered that in these experiments the head of the animal was fixed, so that the only musculature about whose contraction definite observations could be made was the superficial neck musculature: platysma, sterno-cleido, splenius capitis, pinna muscles, etc. The boundaries of these two intermediate regions are drawn in Fig. 2 as more distinct towards the arm-subdivision because we have enough information to establish these margins fairly accurately.

Functional organization of sensory cortex

In the previous work on the macaque's sensory cortex it was found that the distribution of the strychnine spikes following local strychninization of

some area is not only beyond the limits of this area but follows a definite "pattern" for each area. This means, as has been established by further investigations (11), that the various areas of the sensory cortex of the macaque monkey have different interareal neuronal connections. Thus this combination of methods reveals what we have called the "functional organization" of the macaque's sensory cortex

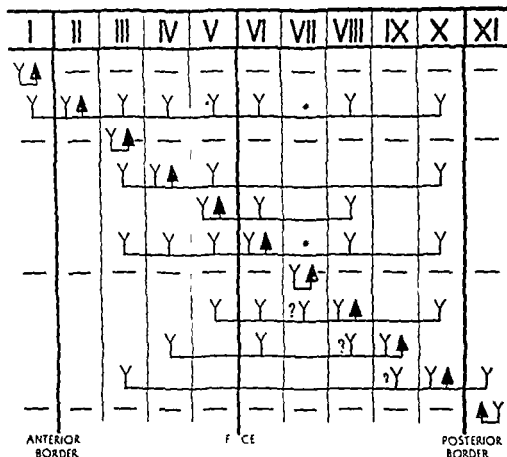


FIG. 4. In Figure 4 are represented diagrammatically the directed functional (and anatomical) relations between the various cortical bands of the arm-subdivision of the sensory cortex found in these experiments and also those of bands I and XI adjacent to but outside of the sensory cortex. Anterior and posterior borders are the limits of the sensory cortex. The suppression of the ECG of various bands upon strychninization of bands I, III, VII and XI is indicated thus: —.

F CE = fissura centralis; * = no certain evidence; ?Y = definite "firing" but uncertainty whether strychnine invaded region so "fired."

The same is true for this cortex of the chimpanzee, but with differences: (i) with truly local strychninization the distribution of the strychnine spikes is in this animal much less widespread than in the macaque, sometimes confined to a relatively small region in the neighborhood of the site of strychninization; (ii) when the spikes are present in another more or less remote region, only a small portion of that region is involved.

Concentrating attention on the arm-subdivision it was found to be composed of several functionally dissimilar vertical "bands" of cortex, characterized by: (i) the unique distribution of the strychnine spikes following strychninization within any one band; (ii) the appearance of strychnine spikes within it following strychninization of other bands.

Unfortunately the only complete cytoarchitectonic map of the chimpanzee's cortex is that of Campbell (3), which fails to show many differentiations found functionally in these experiments. Furthermore, the other available cytoarchitectonic studies on the precentral areas 4 and 6 of the chimpanzee (Brodmann, 1, Bucy, 2) differ so widely from Campbell's findings, as to be of little help here. We have, therefore, abstained from any attempt to correlate anatomy and physiology here and constructed our diagram so as to indicate only the physiological differentiation met in our experiments. We have, therefore, assigned to each functionally unique band so revealed an arbitrary number, I through XI. Bands II through X comprise the sensory arm region, whereas bands I and XI (see below) lie without it. Figure 3 presents the location and extent of these bands, mainly in the arm-subdivision, on the chimpanzee's brain.

While these bands indicate greater differentiation in the chimpanzee's sensory cortex than in the macaque's, there appear certain similarities to the functional organization of this animal's sensory cortex as indicated below. Figure 4 presents the functional organization of the arm-subdivision of the chimpanzee's sensory cortex.

The most anterior band of the chimpanzee's sensory cortex, No. II, like area 6 of the macaque, shows the widest distribution of the strychnine spikes, "firing" practically all of the sensory arm cortex except band IX and being fired by no other band than itself. It should be noted that, like area 6 of the macaque, local strychninization of arm band II "fires" not only the arm-subdivision but also the leg-subdivision and even the face-subdivision. It should further be noted that strychninization of band II also "fires" band I, outside the sensory cortex.

Band III has properties comparable to those of area 4-s in the macaque, in that it gives pure "suppression" of electrical activity of other regions of the sensory cortex and spikes only itself.

Bands IV and V together "behave" functionally like area 4 of the macaque. Local strychninization of band VI results in widespread strychnine spikes in both the pre- and postcentral arm cortex; it does not "fire" band II, or band IX.

Band VII is again a suppressor region comparable to the postcentral suppressor area in the macaque's brain and spikes only itself.

Bands VIII, IX and X together resemble the sensory cortex of the macaque's cortex behind its postcentral suppressor area. Whereas local strychninization of bands VIII and IX respect functional boundaries, this is not the case with band X. Local strychninization of leg X "fires" not only itself but also arm X and leg and arm III, local strychninization of arm X "fires" not only itself but also leg X and leg and arm III.

These are the essential functional similarities of the sensory cortices of chimpanzee and macaque; now as to the dissimilarities.

The outstanding difference is that whereas in the macaque when spiking occurs in an area of the sensory cortex it generally involves all parts of this

area, even though the strychninization is truly local, the spiking upon strychninization within a band of the chimpanzee's cortex is much more restricted even when the area strychninized is much larger. Even with 36 electrodes on the cortex and relatively large strychninizations one cannot hope to obtain the complete distribution of strychnine spikes from a given area in any single experiment. This is not and cannot be represented in Fig. 3 and 4, which are composites of all strychninizations in all 6 animals and show the greatest distribution obtained. What does appear in these figures is the greater differentiation into more bands than areas that could be distinguished in the macaque's sensory arm region, witness the differentiation of the immediate precentral cortex in the chimpanzee, subdivided into bands V and IV, (together comparable to area 4 of the macaque) and the far post-central bands VIII, IX and X. Characteristic of the chimpanzee's sensory cortex is the finding of "firing" of remote regions without "firing" of some intermediate region or regions: the failure of bands II and VI to "fire" band IX, though both "fire" band X; the failure of band IV to "fire" bands VI, VII, VIII, and IX, though it "fires" band X; the failure of band V to "fire" VII, though it "fires" VIII; the failure of band VIII to "fire" band IX, though it "fires" band X; the failure of band IX to "fire" bands V and VII, though it "fires" bands VI and IV, and finally the failure of band X to "fire" bands VIII, VII, VI, V and IV, though it "fires" band III.

An interesting finding is that in the precentral sensory cortex (see Fig. 3) lies a "dud" area, "dud" in this sense, that its strychninization "fires" no other area and that it is "fired" by strychninization of no other area. Above the end of the fissura Sylvii, bulging into the sensory cortex, but not part of it, lies a second "dud" area in the same sense as the first area (see Fig. 3).

Anterior and posterior to the sensory arm cortex were found two bands, I and XI respectively, the local strychninization of which results in a marked suppression of the electrical activity of a great part of the convexity of the hemisphere. Local strychninization of band I results not only in suppression of the electrical activity of the whole arm cortex, but also of that of the leg-, trunk-, neck- and face-subdivisions and of that of band XI; local strychninization of band XI similarly gives suppression of the electrical activity of the entire sensory cortex and of that of band I. It is interesting to note that this widespread suppression from these two areas does not occur simultaneously in all bands of the sensory cortex, but slowly, in the course of many minutes, sweeps across this cortex in a definite sequence, the bands nearest to that strychninized "going under" first, those more remote later. (See Fig. 5 and 6)

Figure 6 gives the right hemisphere of chimpanzee VI on which this particular experiment was performed to obtain the records of Fig. 5 and the position of the electrodes with the changes of the electrical activity of the cortex underneath each of these electrodes (+ for "firing," - for suppression, 0 for no change), and the site and extent of the strychninization. Very interesting is the finding that the sequence of the bands is more orderly

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COLUMNS OF ELECTRODES

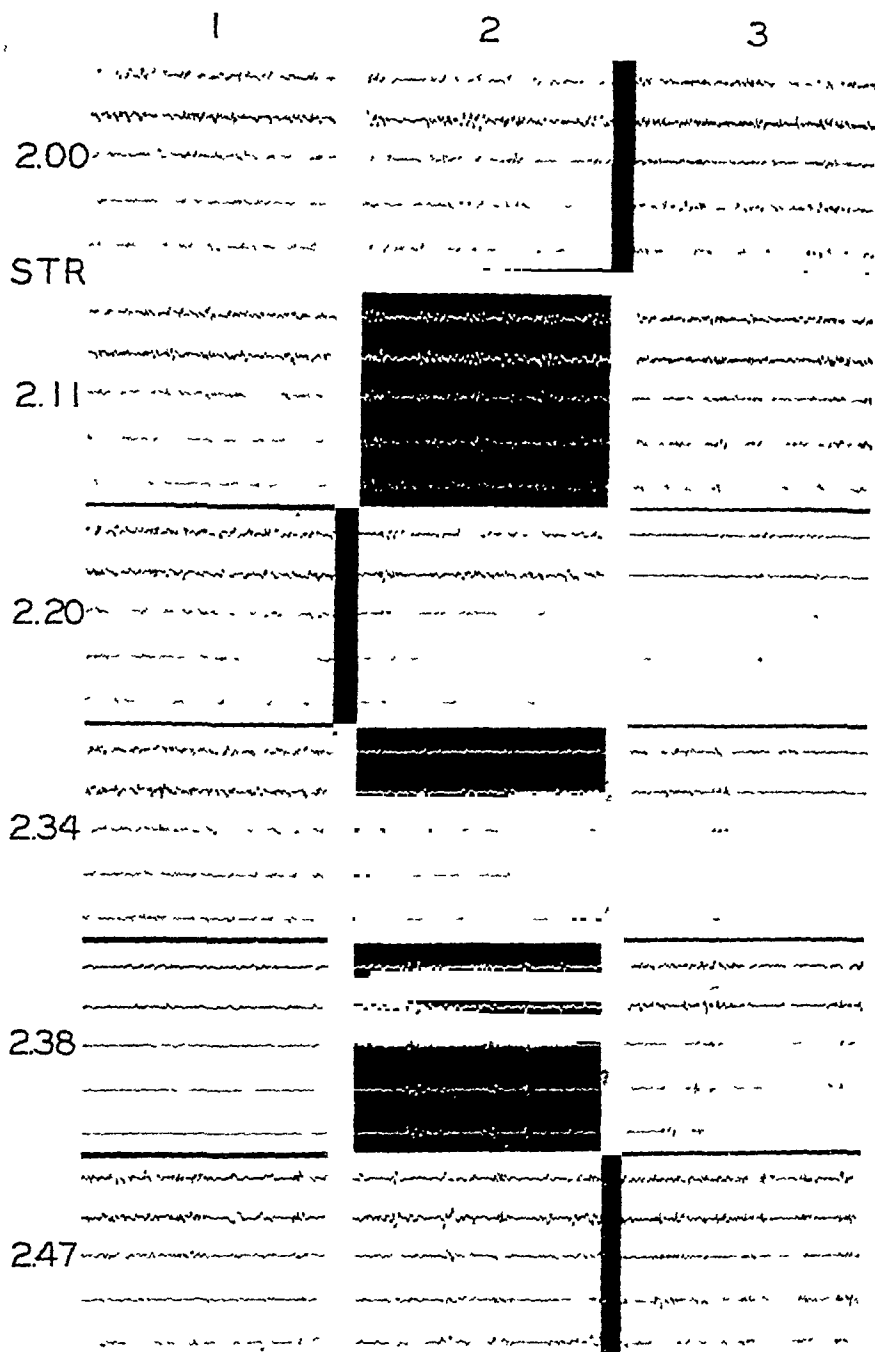


FIG 5. Nov. 7, 1939. Chimpanzee VI. Right hemisphere. Second day. Strychninization No. 18 at S in band XI (see Fig. 6) at 2.04 p.m. between the recording of the ECGs of rows 1 and 2. (For arrangement of electrodes on cortex and on records and their connections with amplifier oscillograph sets see Fig. 6.) Note the "firing" in the ECGs corresponding to electrodes b, c, d and e of column 6, and the "sweep" of the suppression of the electrical activity across the cortex.

SENSORY CORTEX OF CHIMPANZEE COLUMNS OF ELECTRODES

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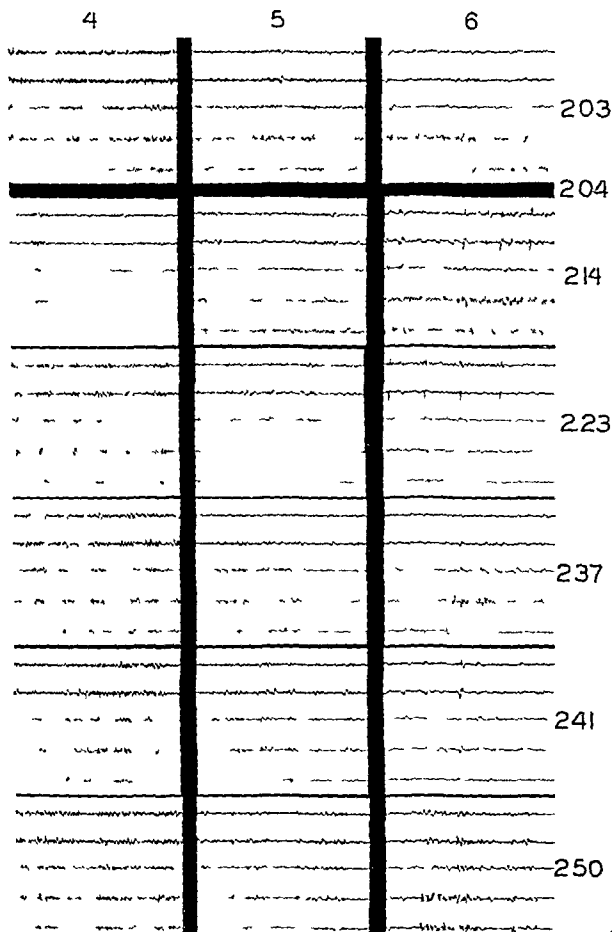


FIG 5 Nov 7 1939 Chimpanzee VI Right hemisphere Second day Strychninization No 18 in band XI (at S in Fig 6 and indicated by STR in Fig 5) at 2 04 p m between the recording of the ECGs of rows 1 and 2 (For arrangement of electrodes on cortex and on records and their connections with amplifier oscillograph sets see Fig 6) Note the 'firing' in the ECGs corresponding to electrodes b c d and e of column 6, and the 'sweep' of the suppression of the electrical activity across the cortex

than that of the sulci, *i.e.*, on crossing a sulcus a band may appear interrupted and one of its portions appear displaced anteriorly or posteriorly depending upon the angle between the band and the sulcus on the surface. For instance, if in a particular brain the "spur" of the first temporal sulcus bends anteriorly, the portion of band XI above this spur lies anterior to its continuation below the spur, whereas in an animal in which the spur of the first temporal sulcus bends posteriorly, the superior portion of band XI lies

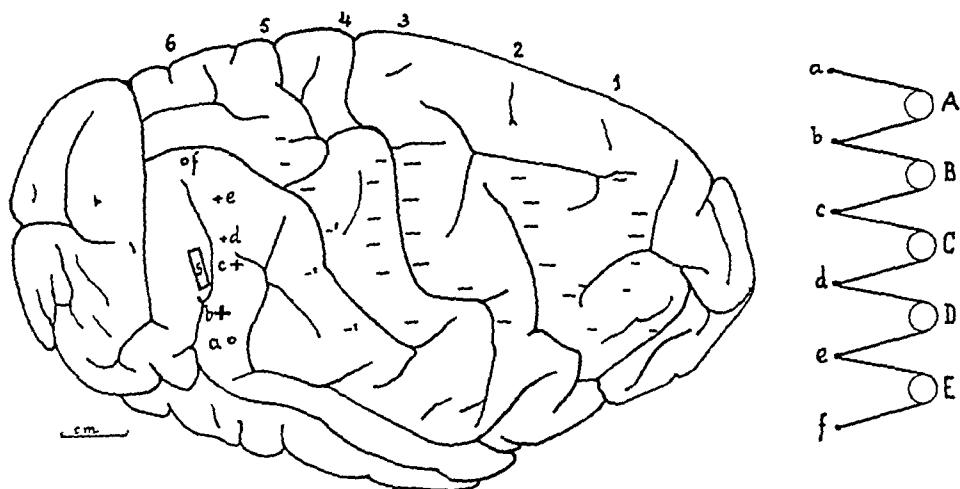


FIG. 6. Right hemisphere of chimpanzee VI with location and extent of strychninization (S), positions and arrangement of the 6 columns of 6 electrodes and the change in electrical activity under each electrode (+ for "firing," - for suppression, 0 for no change). This figure refers to the experiment recorded in Fig. 5. Notice that the arrangement of electrodes on cortex and in records is inverted. a, b, c, d, e and f represent the 6 electrodes of any column, A, B, C, D and E the 5 amplifier-oscillograph sets.

posterior to its inferior, or ventral, portion. This finding must mean that the location of the bands is more fundamental than the location and configuration of the sulci crossed by the bands.

DISCUSSION

The most salient point which these new investigations have brought out, just because of the great amount of information obtained in each hemisphere, is that it is almost impossible to compose a reliable diagram of the location, extent, subdivision and functional organization of "the" chimpanzee's sensory cortex. The variability in the configuration of this animal's cortex is so great as to preclude any definite homologization of any but the principal fissures and sulci. The size, shape, position and direction of the secondary sulci is so variable that these (sometimes, even) often, cannot be identified with certainty. Nevertheless it was necessary to use some composite diagram of the external configuration of the hemisphere. After a careful study not only of the hemispheres in our own collection but also of the available material in the literature (Retzius, Mingazzini and others) the

composite diagram presented in Fig. 2 and 3 was made. This is believed to represent not too unfaithfully the "average" external aspect of the chimpanzee's brain. Since in nearly all of these studies attention was focussed on the arm-subdivision, this diagram was composed from photographs centered upon the middle of this subdivision. Given the great variability of the external configuration of the chimpanzee's hemisphere it will be readily understood that the sites of individual strychninizations and electrodes are extremely difficult to homologize from hemisphere to hemisphere. Although these sites were in each instance plotted on photographs and drawings of that hemisphere on which the particular experiment was performed, it was found most practicable for the final synopsis to transfer them to sites homologized as well as possible on the composite diagram.

The difficulties mentioned are especially great with respect to band VII because it is narrow and occupies the posterior margin of the extremely variable postcentral gyrus. When this gyrus is wide and has a longitudinal sulcus on its convexity band VII is found as indicated in Fig. 3; when the gyrus is narrow it is sometimes impossible to find band VII in the central position of the arm-subdivision and, if found, it may appear immediately behind the postcentral sulcus. Most difficult are those hemispheres in which the postcentral gyrus is narrow and the junction of the superior postcentral sulcus with the intraparietal sulcus is low down, far forward and complicated in form while the longitudinal dimple on the postcentral gyrus is absent or at least does not appear as a separate sulcus. This variability is so great as to make it almost impossible to place electrodes wilfully in this particular band. The diagram of functional organization (Fig. 4) shows, therefore, the most probable changes in activity following the various strychninizations with electrodes probably placed in band VII. The asterisks indicate complete uncertainty in this respect. In chimpanzee 9 we have evidence that if electrodes are placed in the leg region of band VII, strychninization of band II and of band VI "fires" band VII. We are, therefore, inclined to believe that the same is true of the arm-subdivision, where evidence is still to seek.

Similar difficulties arise especially for the more dorsal part of band IX, where it narrows down as it passes into the trunk region, and the variability of the ends of the inferior parietal sulcus, the fissura Sylvii and the first temporal sulcus complicates the picture. However, in the case of band IX enough experimental evidence (sites of electrodes and strychninizations) was available to overcome, in our opinion, the difficulties.

The question marks in Fig. 4 do not indicate uncertainty as to "firing" or position of the electrodes in these bands, but signify that the strychnine may have extended into the region so fired from that adjacent region to which it was applied. In each case the firing marked with a ? was only in the proximity of the strychninization and did not involve more remote parts of the band under consideration.

Not indicated in Fig. 4 are: that in chimpanzee 9 strychninization of

band XI in the leg region, besides "firing" itself, suppressed itself, and that in one instance strychninization in band I, besides "firing" this band, questionably suppressed the same. We shall now discuss the bands seriatim.

Band I. This band lies outside the sensory cortex by the criterion that strychninization within it never "fires" any portion of the sensory cortex. Its primary characteristic is that its strychninization gives suppression of cortical electrical activity, which may involve the entire sensory cortex and even extend beyond it. This suppression does not respect functional boundaries between the major subdivisions nor is it present simultaneously in all parts of the cortex, but sweeps across it from before backward. Thus strychninization of band I may result in suppression of the leg-, arm- and even face-subdivisions, starting in the intermediate precentral cortex, then appearing in the immediate precentral, then in the immediate postcentral and finally in the parietal cortex. The second characteristic of band I is that it is "fired" by strychninization of no part of the sensory cortex except band II. Band I apparently is comparable to that area, lying in front of L. and A. 6 of the macaque's brain, from which eye movements can be elicited and the local strychninization of which gives suppression of the electrical activity within the sensory cortex (15).*

Band II. The primary characteristic of this band is that it is a region, whose strychninization results in the most extensive "firing" obtainable from any region of the sensory cortex. Not only does it "fire" the entire precentral cortex and the entire postcentral sensory cortex, except for band IX, but it also fires band I and fails to respect functional boundaries. For instance: Face II can "fire" even leg X; arm II can "fire" the whole of the arm-subdivision (except IX) and portions of the leg- and face-subdivisions.

It is perhaps an oversimplification to treat band II as a single entity, for in a given hemisphere one can obtain from some portions of band II the "firing" of band X without that of the intermediate regions and by strychninization of another portion of band II "firing" of the intermediate region without "firing" of band X and by strychninization of other portions an admixture of these two results. However, the present evidence does not allow us to make any more definite statements on this score.

Band II is the most anterior portion of the sensory cortex by the criterion used. It is obviously comparable to area 6 of the macaque's sensory cortex, with respect to the distribution of the strychnine spikes, especially the failure to respect functional boundaries.

Band III. This band is a suppressor region, i.e., its strychninization gives a widespread suppression of electrical activity in the sensory cortex and in bands I and XI. The effect on itself is an initial spiking followed by suppression of its electrical activity. The suppression obtainable from this band violates functional boundaries. Its properties and its position immediately behind band II indicate its homology to area 4-s of the macaque's sensory cortex. An apparent difference from area 4-s of the macaque is that,

* In one instance strychninization in band I besides "firing" itself, questionably suppressed itself.

in that animal, we were never able to obtain suppression without "firing" of postcentral areas, whereas in the chimpanzee we have obtained pure suppression in 2 out of 15 cases of suppression from band III. Moreover, the "firing" of some of the regions in the cases of mixed suppression from band III was clearly referable to extension of the strychnine into the adjacent bands.

Bands IV and V. These two bands occupy the cortex between band III and the fissura centralis. In their primary characteristics these bands have in common that their strychninization results in "firing" of other areas, not in suppression. It is, however, necessary to distinguish two bands in this region, band IV and band V, because the strychninization within the frontal portion, band IV, gives a distribution of strychnine spikes different from that following strychninization within the posterior portion, band V, and because the distribution of the strychnine spikes following strychninization of some of the postcentral bands (VIII and IX) is also different in regard to bands IV and V. (See Fig. 4.) The position of bands IV and V is roughly comparable to area 4 of the macaque and a simultaneous local strychninization of the two of them gives results closely comparable to those following local strychninization of the macaque's area 4. Moreover, these bands IV and V are that region of the cortex of the chimpanzee, ordinary faradic stimulation of which elicits prompt, discrete movements, and which cytoarchitectonics pronounces to be the area gigantopyramidalis. Thus, in the chimpanzee it has been necessary to make, in this region, a distinction which thus far we have not been able to discern in the macaque.

Band VI. This band is a "firing" band. Strychninization within this band besides "firing" itself can "fire" bands III, IV, V, VII, VIII and X. We have never observed any "firing" of bands II or IX following strychninization of VI. The "firing" obtainable from this band is the most extensive of the "firings" obtainable from the postcentral sensory cortex.

Band VII. Strychninization of this band results in suppression of electrical activity throughout the entire sensory cortex and even outside—bands I and XI. The electrical activity of band VII itself shows an initial "firing" followed by suppression. In 2 out of 13 cases pure suppression was obtained.

Band VIII. This band is a "firing" band, affecting of the precentral cortex only band V.

Band IX. This again is a "firing" band, which can be differentiated from band VIII in that its strychninization does not "fire" band V, but "fires" band IV. With respect to the postcentral cortex, it should be noted that band IX does not "fire" X, whereas strychninization of band VIII does "fire" X. Moreover, band IX is unique in the postcentral sensory cortex insofar as it is "fired" only by strychninization within it. The question mark re "firing" of this band from band X denotes uncertainty whether the strychnine responsible for this "firing" of the latter band had not transgressed into band IX.

Band X. Again this is a "firing" band, strychninization of which "fires"

only band III of the precentral cortex and, of the postcentral sensory cortex, only itself. This band of the postcentral sensory cortex is unique in that its "firing" does not respect functional boundaries, *i.e.*, does not remain within the subdivision locally strychninized. Strychninization of leg X can "fire" leg X and arm X and leg III and arm III. It also "fires" band XI, outside the sensory cortex. We say "outside the sensory cortex" because band X being the most posterior region to "fire" into the sensory cortex, by this criterion, is the most posterior of its constituent bands. With respect to homologizing the postcentral bands with the postcentral areas of the macaque's sensory cortex we wish to take up the discussion of these bands together, since we have not as yet been able to make a comparable differentiation in the macaque. All we can say definitely at present is that in both the chimpanzee and the macaque there exists a postcentral sensory suppressor band—band VII of Fig. 3 and 4—behind which lies a region which in both animals "fires" into the precentral sensory cortex. In view of the findings in the chimpanzee the functional organization of the sensory cortex of the macaque's brain is under reinvestigation with cytoarchitectonic controls. Not until this work is completed, do we dare to say anything in regard to homologization of the more anterior parts of the postcentral sensory region of the chimpanzee and macaque.

Band XI. This band lies outside the sensory cortex by the criterion that strychninization within it never "fires" any portion of the sensory cortex. Its primary characteristic is that strychninization within it suppresses the entire sensory cortex and even band I in front of it.

For the arm-subdivision we have no evidence that strychninization of band XI besides "firing" itself may also later suppress itself; for the leg-subdivision (chimpanzee 9) the sequence of these two phenomena was observed.

In the re-investigation of the macaque's cortex mentioned above special attention was paid to the question whether in this animal a region outside the sensory cortex, comparable to band XI of the chimpanzee's brain, exists. The investigation has proceeded far enough to show that this is the case and to state that these bands in the two species correspond closely as to position and shape. This statement holds also for the manglebe's brain.

It has been found that the posterior border of the sensory cortex in the chimpanzee and, with it, the location of band XI on this brain in relation to the fissura parieto-occipitalis externa and the end of the first temporal sulcus shows great variability. In the chimpanzee on which the diagram of the sensory cortex in the preliminary note (Fig. 1) was based this cortex extended clear to the parieto-occipital fissure. In all the animals upon which this paper is based it was found that the posterior border of the sensory cortex lay in front of this fissure and even that in some hemispheres band XI—outside the sensory cortex—did not extend as far back as this fissure. The same variation has subsequently been found in the macaque. These findings suggest that band XI and its homologue in the macaque may well be area 19 of Brodmann.

Interesting is the posterior "dud" area bulging into the sensory cortex between the end of the fissure of Sylvius and the first temporal sulcus. The diagrams of Campbell (4) for man and the subhuman apes show a region of essentially similar configuration, belonging, according to Campbell, to the temporal lobe bulging into the parietal cortex, the posterior end of his "audiotopsychic" area. We wish to point out that it was not until we were writing this discussion and trying to make the homologizations that we became aware of the remarkable coincidence between this point in our physiological findings and Campbell's histological studies. A similar "dud" area has subsequently been found in the macaque in the vicinity of the end of the fissura Sylvii and anterior to the first temporal sulcus.

In conclusion it should be emphasized that the diagram of the functional organization of the chimpanzee's sensory cortex as given in Figs 3 and 4 shows the maximum of "firing" and suppression observed, not in one animal nor following one strychninization, but that it gives the synopsis of all data collected.

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UNIT FOR SENSORY RECEPTION IN CORNEA

WITH NOTES ON NERVE IMPULSES FROM SCLERA, IRIS AND LENS

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WITH THE ADVENT of unitary analysis of activity in the nervous system, heralded by Adrian and Bronk's (1928) paper recording the impulses carried by single fibers isolated in peripheral nerves, a method became available whereby the terminal distribution of a single afferent fiber might be disentangled from those of its associates, identified as a unit, and so examined. This terminal, together with its fiber, cell body and central processes, could be considered the sensory equivalent of the neuro-motor unit. Nevertheless the basic problem: what constitutes the functional unit for sensory reception: still remains largely unsolved.

To review the perplexities: When a single nerve fiber terminates in a single encapsulated ending, Pacinian corpuscle, muscle spindle, or other, the nerve tissue within the encapsulation clearly comprises the entire receptive terminal of the neuron. This, the simplest, is also the rarest case, but the innervation of some of the frog's spindles examined by Matthews (1931) probably conformed with this design. When, however, a single fiber innervates two or more such corpuscles or spindles, what is to be considered the unit nerve ending: all the terminal tissue of one nerve fiber or the separately encapsulated portions of terminal tissue? If the multiple end-organs are dissimilar, as may be the case (Hines and Tower, 1928; Woollard, 1936), qualitative as well as quantitative considerations arise. When two or more nerve fibers enter into one encapsulated end-organ, the problem presents three possibilities. The separate fibers may derive from the same fiber farther back. If not, the one may be afferent and the other efferent. If both are afferent, the problem remains theoretically simple if the two neurons remain separate. The end-organ may be then considered compound, and suspect, as in the case of some mammalian spindles, of serving diverse sensory functions. If, on the other hand, the two neurons fuse in their terminations, a condition which has been often suspected but never unequivocally proven, then from a physiological standpoint at least the problem is complicated indeed.

Still more difficult of definition, however, is the organization in unencapsulated sensory innervations. In these, nerve fibers enter into plexuses or ramifications, perhaps with terminal knobs or loops or small skeins, to be distributed over unascertained areas. Such ramifications are the most widespread form of afferent innervation of both skin and viscera, and probably the phylogenetic prototype. Yet unless by the fluke which gives a complete Golgi impregnation of one nerve cell body and its processes standing out in the midst of its less stained neighbors, histological techniques will be severe-

ly taxed to demonstrate the neuron units in such organization if the unit is distributed over more than a few square millimeters. A plexiform arrangement of nerve fibers may be traced over a large area by silver, or better by methylene blue staining, but the individual neurons can scarcely be kept track of. This obscurity of the neuron unit has fostered a tendency on the part of anatomists to consider the so-called free nerve endings in terms of a terminal reticulum or network in which syncytial continuity of individual neurons is at least suspected. Yet such a viewpoint is as unfounded on anatomical fact as the contrary supposition of individual discreteness, and considerably less acceptable *a priori* because of the validity of the neuron doctrine at the outset of the embryological development of this afferent innervation together with the rest of the nervous system.

Since the problem became open to examination by physiological procedure, however, the neuron unit in such a ramification has been delimited in three sites, with no indication in any that the units are other than discrete. Adrian, Cattell and Hoagland (1931) have outlined on the frog's skin areas of terminal distribution of single afferent neurons sensitive to touch which Rubin and Syrocki (1936) later identified with free nerve endings in the epidermis. The areas defined ranged from 4 to 100 sq. mm. but actually, since the demonstration depended on bifurcation of sensory axons close to the dorsal root ganglion, the two branches then diverging into different divisions of the spinal nerve, the terminal field outlined for one branch was presumable only a portion of the fiber's total field. In the frog's viscera the terminal distributions of single afferent fibers have been worked out by myself (Tower, 1933). These were surprisingly large, often covering 2 or 3 sq. cm. and not infrequently twice that area. In contrast, the fields of presumed touch fibers in the cat's tongue examined cursorily by Pfaffmann (1939) were fairly small, ranging around 5 mm. in diameter.

The mammalian cornea exposes for examination an uncomplicated field of the ramifying or plexiform type of innervation. The nerve fibers entering into this innervation, all of which is considered, though without proof, to be afferent, are in large part myelinated, but lose their myelin sheaths as they pass from the periphery toward the center. The fine unmyelinated ramifications of these fibers form a plexus in the connective tissue with terminal twigs or knobs or loops or brushes or fine skeins, and with branches penetrating into the epithelium where they may form a second plexus. Within these plexiform arrangements of fine unmyelinated nerve fibers, which have been described and illustrated by Dogiel (1890), Attias (1912), Boeke (1935) and others, there is no anatomical cue to the neuron units. Except close to the sclero-corneal junction, no encapsulated endings are present. The cornea thus invites the attempt to outline the neuron units in a terminal ramification by physiological procedure, and to attack the problem of the discreteness or confluence of these. To this end arrangements were made to amplify and record action potentials in the long ciliary nerves of cats in conjunction with stimulation of the cornea. Ultimately the investigation was extended to other parts of the eyeball.

PROCEDURE

The cats were decerebrated, one orbit unroofed, the 2nd to 6th cranial nerves cut extracranially and the ciliary ganglion removed. The long ciliary nerves were freed from the surrounding tissue and cut as far centrally as possible, often yielding more than 2 cm. for lead. The eyeball was left in its bed after trimming off the conjunctiva at its reflection from the eyeball. To interfere with circulation as little as possible, both vertebral arteries and the carotid of the side under investigation were occluded usually only during the decerebration. The other carotid was ligated. To fix the eyeball, a fringe of conjunctiva was either sewed to a ring fitting the corneal margin; or a perforated button was inserted behind the cornea through a slit along the sclero-corneal junction, with or without the ring lightly clamped in front. Such a button must be removed at frequent intervals to allow free irrigation of the posterior surface of the cornea. Often no device was used. Throughout the experiment the animal was kept in a warmed and humidified metal cage which served also as electrical shield.

For stimulation the following were employed: a set of von Frey needles ($\frac{1}{8}$ to 10 gm.); a corresponding set of hairs (2 to 20 gm. per sq. mm.); a mechanical stimulator delivering the prick of a cactus spine with variable intensity calibrated in grams and with 3 dimensional spatial control; and blunt glass rods. Pinching and tearing were also resorted to.

The action potentials were led off through silver, silver-chloride, ringer, brush electrodes to a condenser-coupled amplifier and Matthews oscillograph, and photographic records were made.

The long ciliary nerves were used entire to lead from when fine filaments were present or when thick, reduced by about half. In a number of experiments, however, the active fibers of one of these nerves were reduced to two or three, and for this skillful manipulation I am indebted to Dr. D. W. Bronk. These will be referred to as "few fiber preparations." For the gross anatomy of the cat's long ciliary nerves, which is extremely variable, reference may be had to Christensen (1936). According to Windle (1926) they are composed of non-myelinated and small myelinated fibers all under 7μ in diameter. Anticipating the simplest results of the present study: Of the two main divisions of long ciliary nerves the medial supplies the medial and inferior portions of the cornea and the lateral the lateral and superior portions, with overlap of several millimeters at the center and along these territorial boundaries.

OBSERVATIONS

The nerve impulses from receptors in the cornea constituted a graded series from moderately large to quite small. To what extent this apparent gradation was a function of fiber size, to what extent of more or less favorable situation at the lead, it is impossible to say. Of necessity attention focused on the larger members of the series, the smaller being progressively lost track of as they approximated to the instability of the base-line. All the activity which is the material of this report took the form of spikes in the records. By comparison with Zotterman's (1939) records from stimulating the cat's skin, purporting to show activity in unmyelinated fibers, all these spikes presumably represented activity in myelinated nerve fibers.

Leading from fine filaments of long ciliary nerves the responses obtained from the cornea using the different forms of mechanical stimulation were essentially similar: trains of impulses of rapidly diminishing frequency, or occasionally with threshold stimulation a single impulse only. Initial frequency and duration of the discharge were functions, both, of the intensity of the stimulus. Thereafter with continued stimulation the discharge diminished, sometimes to cease entirely in a few seconds, sometimes to establish a level of activity sustained for as long as the stimulation. With all but the lightest stimuli, removing the stimulus commonly caused a second small

outburst. Not infrequently, when the number of fibers responding was small and the individuals identifiable, certain fibers adapted completely while others did not, to the same stimulus. The $\frac{1}{8}$ gm. von Frey needle was only once effective, the $\frac{1}{4}$ rarely, the $\frac{1}{2}$ gm. usually; but threshold might be as high as 1 gm. in a preparation which gave no other sign of deterioration. The hairs yielded effects similar to those obtained with the needles except that threshold was rarely less than 4 gm.

Isolated by attack on the nerve, one nerve fiber of the type yielding fairly large impulses was found to be distributed over roughly a quadrant of the cornea and usually over the adjacent 1 to 3 mm. of sclera. Certain fibers, those with the largest spikes, covered an even larger territory, very nearly one half the cornea and the adjacent sclera back almost to the equator of the eyeball. When a fringe of conjunctiva remained, fibers of corneal distribution were also found distributed there, but the extent of such spread was not worked out. Within this large area of 50 to 200 or more square millimeters were islands not supplied by the fiber in question, but perhaps in a two fiber preparation by the second of the survivors. The lowest threshold and slowest adaptation were usually 2 or 3 mm. inside the sclero-corneal junction in the midst of the fiber's field, and both increased irregularly toward the periphery in all directions. Rapid adaptation even to the limit of a single impulse characterized the periphery more than did high threshold. Considering the large area of distribution of the single fiber and the slight deformation produced by all except the strongest stimuli, only near the periphery would the deforming action overstep the limits of a fiber's field. Therefore the greater central sensitivity must represent either a greater central concentration of nerve-ending tissue, or more strategic situation, or degradation from center to periphery of the excitability of the nerve tissue itself. Fibers carrying the smaller impulses seemed to have more restricted distribution, but their identification as individuals was never so certain as with fibers of larger impulse size.

Threshold for the single fibers covered the same range as threshold for a whole nerve. Frequency, on the other hand, was usually very much less. Yet for the first hundredth second of response to a fairly strong stimulus, frequencies in excess of 500 per sec. are on record for unquestionably single fibers; and to moderately strong stimulation such as the 4 gm. needle, frequencies of 220 per sec. for the first fiftieth second; while the 2 gm. needle commonly evoked 3 impulses in the first fiftieth second. At threshold and up to twice threshold, however, the discharge for the first one-fifth second was fairly constant, and rarely in excess of 40 per sec. In the next two-fifths second it dropped off, either to cease entirely, or to establish a level of continued discharge at 1 to 8 per sec. After the stimulus was removed, a corneal ending which had not previously been spontaneously active showed no tendency to continue discharging.

Rate of adaptation was a function both of the site and of the intensity of stimulation. Adaptation might be complete after the discharge of one im-

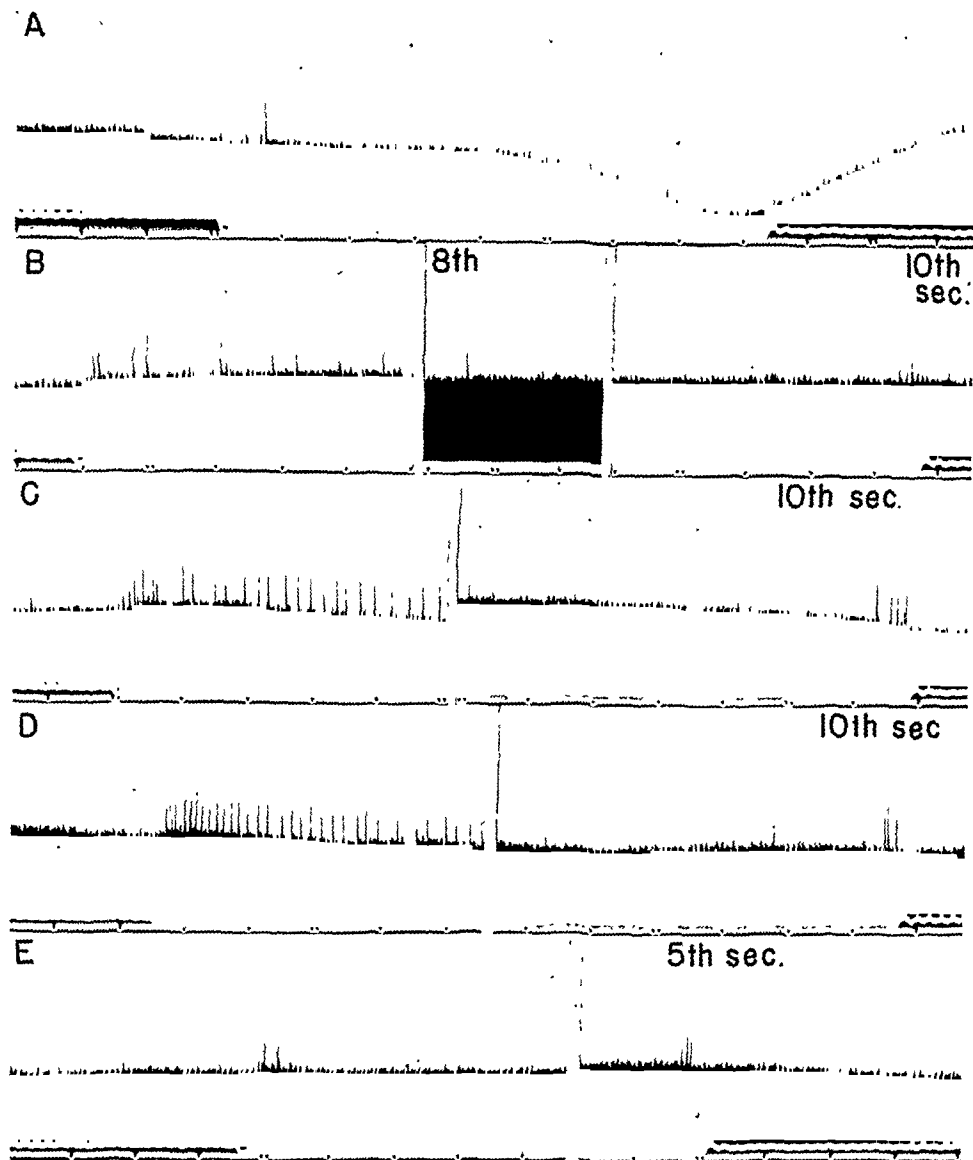


FIG. 1. Response, adaptation and fatigue to a series of stimulations of increasing strength applied by the mechanical stimulator to the most sensitive spot in the corneal field of a fairly large fiber. "Few fiber preparation" of a lateral long ciliary nerve. Duration of stimulation shown by upper signal; time in 0.2 sec. by lower.

a, contact: b, 1 gm. for 10 sec. last response in the 8th sec.; c, 4 gm. for 10 sec. last response in the 9th sec.; d, 8 gm. for 10 sec. adaptation still incomplete; e, 1 gm. for 5 sec. adaptation almost at once.

pulse only with any effective stimulation on the periphery of a fiber's field, or with contact in the most sensitive central area (Fig. 1A), whereas with stronger stimulation in this area it might require ten seconds or longer

(Fig 1B-D) In the central area of a fiber's field, beginning at threshold and increasing the intensity of stimulation increased the duration of discharge more conspicuously than the initial frequency (Fig 1A-D), that is, it slowed adaptation. Both deterioration and fatigue operated faster and more effectively to accelerate adaptation than to reduce the initial response. Though clearly illustrated in Fig 1 by comparison of B with E, this effect of fatigue was even more striking in a longer series of 100 stimulations at 1 gm, 1 sec on and 2 sec off. Throughout such a series the initial burst retained very nearly its original proportions, but adaptation speeded up until it followed immediately on the initial burst. Series timed with the periods of rest and of stimulation approximately equal exhausted the initial response as well as the continued. For the central regions of active fibers and for equivalent stimulation, adaptation was always slower at the start of an experiment than toward the end. These observations create the impression that the normal corneal sensory mechanism is characterized by slow or incomplete adaptation in the central most sensitive portions of the terminal distribution of individual nerve fibers, in contrast with exceedingly rapid adaptation on the periphery and in the most superficial ramifications.

Within the terminal ramification of one nerve fiber local conditions influenced both the initial frequency and the duration of discharge evoked by a given stimulation. Denial of access of aqueous humor to one part and not to another raised threshold and speeded adaptation locally. This was brought out by the use of buttons with various sized perforations to back the cornea, for the cornea over the solid parts rapidly became unresponsive while that over the holes remained active. The gradual deterioration with time also attacked the ending irregularly, though with a usual progression from the center of the cornea toward the sclera. At best, however, adjacent parts of a fiber's field were rarely equally responsive. From point to point the discharge varied, though always with a general gradient of decreasing activity from a central most sensitive region toward the periphery in all directions.

Adaptation and fatigue were also spatially restricted processes in healthy preparations. Thus a series of stimuli which rapidly produced fatigue or cumulative adaptation if directed to one point, failed to do so if spaced a millimeter apart. With continued moderately strong stimulation to which adaptation was complete the adaptation affected such a limited area that a second stimulus as close as possible, perhaps a millimeter, from the site of the first, provoked a fresh vigorous discharge over the same nerve fiber. The mechanical stimulator was used for the continued stimulation, a von Frey needle for the intercurrent.

The separate regions of one nerve terminal were nevertheless not completely independent, on the contrary the whole terminal appeared to be conditioned by each of its parts. From time to time preparations were obtained in which a nerve fiber with corneal distribution was spontaneously and rhythmically active 1 to 8 times per sec. sometimes for hours. Such

"tickers" were readily identifiable as individuals when the nerves were cut down, although leading from a whole nerve, their individuality tended to be submerged. One three-fiber preparation had two such "tickers," one ephemeral, the other performing for 8 hours at a rate falling from 5 to 7 per sec. at the start to 1 to 4 per sec. at the end. This latter is the fiber active in Fig. 2. Such spontaneous discharge was never absolutely regular even over very brief periods of time, and was subject to the same conditions as deliberately excited action, slowing and ceasing when the circulation of blood or aqueous humor was interfered with, or examination too long continued, and being restored by rest and care. Although this indicates a corneal origin for the spontaneity, which was verified in two instances by excising the cornea, whereupon it ceased, deliberate attempts to provoke spontaneity by damaging the cornea were not successful.

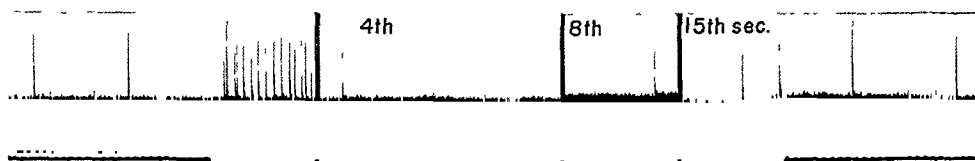


FIG. 2. Spontaneous activity in a fiber with corneal distribution, and inhibition of this by 4 gm. stimulation applied with the mechanical stimulator to the central region of the fiber's terminal. Inhibition was complete from the end of the 3rd to the middle of the 8th sec., and the control rate of spontaneous discharge was reestablished only in the 12th sec. Few fiber preparation of a lateral long ciliary nerve. Duration of stimulation shown by upper signal; time in 0.2 sec. by lower.

Stimulation of the corneal terminals of such a "ticker" elicited the usual outburst of impulses, but following this, either after the stimulus was removed or after adaptation to continued stimulation, the spontaneous firing temporarily slowed or ceased. With strong stimulation which was not adapted to, the depression of spontaneity became evident only after the stimulus was removed. In one record the spontaneous firing regained the control value only 25 sec. after the "off," while suppression for 5 to 10 sec. was frequent. Figure 2 summarizes such inhibition of 4.5 sec. duration resulting from moderately strong stimulation; while a more concise record of a briefer instance has already been published (Tower, 1935) in a preliminary report. Stimulation anywhere within the extensive terminal field of the spontaneously active fiber produced the depression and in proportion only to the volume of the discharge called forth, while excitation of other fibers within the area had no such effect. The inhibition was most active with the preparation in best condition, being then recorded from as little as two stimulated impulses. It was certainly effective, that is as outright inhibition, only against the presumably minimal excitation which fired spontaneously and not against a stimulus such as the 1 gm. needle applied in the inhibitory period, though a small reduction of response to such a stimulus would scarcely have been appreciated. In the opposite direction, on rare occasions toward the end

of an experiment when a "ticker" was firing feebly and irregularly, weak stimulation in the fiber's field appeared to increase and to steady the spontaneity, and sometimes even to reinstate it after it had lapsed.

The inner surface of the cornea, exposed by a cut along the sclero-corneal margin in its upper half, responded on stimulation applied as to the outer surface with a discharge of similar characteristics, but of lesser volume.

DISCUSSION

By isolation of nerve fibers in the nerve trunks the terminal ramification of the single sensory fiber to the cornea stands out as a unit distributed over 50 to 200 or more square millimeters of cornea and adjacent sclera and conjunctiva. The sharpness of outline of the single fiber's field, the occurrence of islands with other innervation within that field, and the different patterns of terminal distribution of individual nerve fibers all testify to the composition of the total terminal plexus as an aggregate of units and against the existence of a syncytial terminal nerve net entered into by numbers of fibers. Meshwork of the order of magnitude figured by Boeke (1935) for example, 100μ more or less in diameter, must therefore represent structure within the total ramification of the unit neuron.

If the terminal units thus outlined on the cornea and sclera include all the terminal tissue of one nerve fiber, then they constitute together with their cells and fibers, neuro-sensory units on a par with the neuro-motor units. But only by leading off immediately adjacent to the root ganglion cell and exploring the entire field of distribution of the Vth nerve could the possibility of axon bifurcation be excluded, and not in a lead from the long ciliary nerves. Such axon bifurcation, besides providing the basis for Adrian, Cattell and Hoagland's (1931) experiment outlining the fields of touch fibers in the frog's skin, almost certainly underlay Wernöe's viscerocutaneous reflex in the fish (1925), and naso-ocular vasodilator reflex in man (1922), while Lewis (1936) has presented evidence which can be interpreted only on the basis of similar bifurcation of axons in his nocifensor system in man. Within the maxillary division of the Vth nerve, for example, a single afferent neuron thus appears to have terminal ramifications both in the mucous membrane of the antrum and in the skin of the face. With this reservation in mind, however, and with the further reservation that the known spread of some fibers onto the conjunctiva was not fully explored, the terminal units outlined for the cornea represent neuron units of afferent innervation.

The question still remains, however, what constitutes the functional unit for sensory reception,—each of the numerous small terminals described by the anatomist on one nerve fiber, or the neuron unit of terminal ramification? The evidence presented shows that adaptation is quite localized within the terminal ramification, together with fatigue and other conditions influencing general responsiveness. Yet some aspect of the receptive activity, presumably the propagated impulse, seems to spread throughout the terminal ramification to alter excitability elsewhere than at the site of original

stimulation. With point stimulation, although the mechanical deformation can scarcely act on more than a small fraction of any neuron unit terminal as determined by the site and strength of the stimulus, still when mechanical stability was achieved the train of impulses was quite regular in the individual fiber with all strengths of stimulation, making it unlikely that parts of the unit were firing off independently.

Regulation whereby haphazard firing may be prevented, and the train of impulses evenly spaced with a frequency conditioned both by site and by intensity of stimulation can be conceived in terms of structure. Adrian (1932, p. 29) made the suggestion that rapid and slow discharges may not arise from the same point; that there may be a gradual transition from nerve fiber to nerve ending with a gradual slowing of time relations, and that an intense stimulus may take effect at a point where recovery is rapid. Translating this into morphological concept: If the unit terminal is an arborization wherein a stem fiber, which may or may not be myelinated, divides into branches successively finer out to the ultimate free end or closed mesh, with or without mesh connections in the previous orders of branching, then a strong stimulus near the point of entrance of the nerve fiber into its terminal ramification might be expected to push frequency to the limit permitted by the absolute refractory period of the fiber itself, and even to infringe on the relative refractory period. The maximum frequency of 500 per sec. in the response from the cornea suggests such limitation. In support of this view highest frequency, lowest threshold and slowest adaptation were usually found in individual fiber terminals, not at the center of the cornea, but a few millimeters inside the sclero-corneal junction where anatomical studies have described the myelinated nerve fibers as losing their myelin sheaths and commencing to ramify. Since the nerve fiber always begins to arborize fairly deeply, the deformation of a feeble stimulus at that site could not be expected to reach the trunk fiber, but only the more strategically situated and finer superficial ramifications. If fineness imposes slowness, the slow train in response to weak stimuli may be thus determined. On the periphery of a large field, many millimeters from the point of entrance of the nerve fiber, intense stimulation could be expected to affect only the outlying parts of the arborization, again presumably finer and of slower properties. And correspondingly, on such a periphery in the cornea, stimulation, no matter how strong, provoked a brief, widely spaced train of impulses or one impulse only, like threshold or contact stimulation in the center of the field. If the individual arborization becomes intra-epithelial both superficially and on its periphery, a point on which there is as yet no anatomical evidence for the cornea but extremely suggestive evidence for the skin (Woollard, Weddell and Harpman, 1940), this might explain the similar rapid adaptation in the two sites, in contrast with the slow or incomplete adaptation to strong stimulation in the central area. For Hoagland (1936) has related the rapid adaptation of the frog's touch fibers, by comparison with the slow adaptation of deeper lying fibers which presumably serve pain, to the rapid liberation of

large amounts of potassium by deformed epithelial cells and not by the deeper tissues when similarly stimulated. In conformity with this view is the recent agreement of Waterston (1933) and Woollard (1936; see also, Woollard, Weddell and Harpman, 1940) for the human being, that touch is the dominant sensation obtained from the epidermis whereas pain is lodged mainly in the subepidermal levels.

Putting the facts together, the sensory receptor in the cornea emerges as all the terminal tissue of one nerve fiber. This is a unit, activity in any part of which affects the whole. Moreover there is no evidence that activity in this unit influences in any way the activity of spatially coextensive units. Functionally, the corneal sensory mechanism appears as an aggregate of units and not as a continuum. Nevertheless within the unit there are possibilities of correlated structural and functional differentiation such that the frequency and duration of the train of impulses conducted to the central nervous system may be determined not alone by the intensity of stimulation but also by the site. This introduces a new condition into the central evaluation of peripheral stimulation which may permit of central analysis of peripheral locus on other than a one site: one fiber relationship. By central analysis of a pattern of excitation wherein fibers excited minimally encircle fibers more strongly excited, a crude localization may well be achieved, yet the volume of the response from the encircled fibers, or better, the frequency of the discharge in the individual fibers most strongly excited, still serve to signify the intensity of the stimulation as has previously been assumed.

The central portion of the human cornea yields to any form of stimulation, only one sensation. With more than threshold stimulation this is unquestionably painful. Contrary to von Frey's (1894) contention that the sensation is wholly painful, Goldscheider and Brückner (1919) have presented convincing evidence that beneath the pain there is also a feeling of touch or pressure, not as a separate modality but as a second aspect of one sensory experience. The reality of this touch has been clearly exposed recently by the employment of Sjöqvist's operation of tractotomy (cutting the descending root of the Vth nerve in the medulla) for relief of trigeminal neuralgia. Rowbotham (1939) examined two such cases and established that, although the cornea was insensitive to pain, the contact of a wisp of cotton or of a camel hair brush was appreciated as touch. This has been confirmed by Grant, Groff and Lewy (1940). The bifurcating fibers of the Vth nerve become intelligible in this relationship, with their branches terminating respectively in the main sensory (touch) and descending (pain) nuclei of the Vth cranial nerve. The cornea is not, however, peculiar in this conjunction of a touch quality and pain. Heinbecker, Bishop and O'Leary's (1934) study of cutaneous sensibility described prick pain as the affective quality resulting from more than threshold stimulation of a pricking-touch sense. This threshold pricking-touch and its more than threshold pain they distinguished both from familiar modality of touch and from the pain of hot and burning. Correspondingly, Nafe and Wagoner (1937) showed that the central portion

of the cornea which is insensitive to warmth and cold, is also insensitive to the pain of excessive temperature stimulation. Thus sensation from the human cornea seems to be unified in a touch-pain continuum.

The reactions of laboratory mammals to stimulation of the cornea recapitulate those of man. Therefore it may be inferred that the sensation evoked is likewise dominated by pain. The wide extent of the single ending, covering a square centimeter or more, reflects one subjective characteristic of pain sense wherever this is found devoid of specialized touch as in the cornea, glans penis or viscera,—its lack of any but the most general localization. Likewise the initially rapid but then often incomplete adaptation repeats another aspect of the same subjective experience. Bearing in mind Heinbecker, Bishop and O'Leary's (1933) experience with electrical stimulation of human peripheral nerves, that a stimulus of strength more than adequate to call forth pain on repetition, applied as a single shock registers, not as pain, but as prick: the single impulse or very brief train which results from minimal or peripheral stimulation of the cornea, arising perhaps in terminal tissue strategically located in the epithelium, may be suspected of registering as the threshold or background touch, whereas the higher frequency discharges from deeper reaching deformation may certainly be construed as pain.

Conscious painful experience, however, presents two aspects, stimulus pain as from a prick, and the persistent dull ache or pain of injured tissue. Previous attempts to account for the latter phenomenon (Adrian, 1932; Zotterman, 1939) have invoked the unmyelinated pain conducting C fibers. Yet the records from stimulating the cat's cornea are lacking in evidence of very slow impulses and waves such as Zotterman clearly obtained from the cat's skin and ascribed to activity in C fibers,—synchronized in the waves. If this may be taken as evidence that non-myelinated fibers have small share in the innervation of the cornea, and the anatomical descriptions of that innervation certainly dwell largely on myelinated nerve fibers as the source, then injury pain must derive from properties of pain endings in general and not from peculiarities of the C fibers alone. In the "tickers," fibers of demonstrated corneal distribution, the steady discharge of 2 to 8 impulses per sec. over a period of hours, a discharge, moreover, which does not prevent response to interpolated stimulation, is just such an activity as might be expected to underlie a continuous dull ache or pain. Thus the properties of the sensory mechanism studied in the cornea, seem to offer peripheral determinants of the central processes resulting in the sensation of pain.

Notes on nerve impulses from receptors in other parts of the eyeball

Sclera. By comparison with the cornea, the sclera posterior to the reflection of the conjunctiva was poorly supplied with nerve fibers gauged by the volume of response to equivalent stimulation, but the form of response to pressure, prick, pinching and pulling was similar everywhere over both cornea and sclera. Over the anterior part of the sclera, the fibers were in

large part those distributed also over the cornea, but each site possessed fibers not distributed to the other. Figure 3a shows a stimulation of the sclera in the region of the equator in which the dominant fiber responding was not known to have other than scleral distribution. The sensibility of the sclera was not appreciably altered by cutting off the cornea and evacuating the entire contents of the eyeball: vitreous, lens, iris, ciliary body and retina; but the more violent of the stimulating procedures applied to the sclera, and perhaps all of them, may very well have involved the chorioid also. Agababow (1904) may be consulted for the morphology of the afferent innervations of sclera and chorioid in which the freely ramifying fiber again predominates.

Iris. In contrast with the sclera, the iris, exposed by excising either the upper half, or the entire cornea, proved to be exceedingly sensitive. Leading from a fine nerve, the slightest push on the iris with a light von Frey hair, or pull by means of a thread passed through its margin provoked a furious outburst of spikes in which the characteristics of the constituent impulses were obscured. With the lead cut down so that the discharge from the iris was sufficiently scanty to permit examination, the range of impulse size proved to be similar for iris and cornea, but with a predominance of medium to small spikes. Figure 3b illustrates such a discharge.

Lens. By carefully trimming out the iris with a fine scissors, the lens was exposed *in situ*. While not demonstrably sensitive to prick or to touch with the von Frey needles or hairs, pushing on its anterior surface by means of a blunt glass rod provoked a small outburst of good sized impulses interspersed with a few smaller impulses, the total representing activity in less than half a dozen fibers. In this small discharge, which is illustrated in Fig. 3c, repetitive action of individual fibers was easily recognized. Traction on the lens by means of a thread passed behind it produced a more confused series of medium and small impulses, again of brief duration. Altogether, the lens appeared to be without surface sensibility, but to possess what might be characterized as proprioceptive sensibility in modest proportions. However, since no nerve fibers reach the lens so far as is known anatomically, the receptors in question probably were located in the ciliary body at the attachment of the subtenacular ligament.

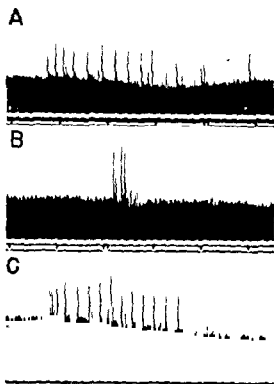


FIG. 3. a, Response to stroking the sclera at the equator of the eyeball with a blunt glass rod. "Few fiber preparation" of a lateral long ciliary nerve. Time 0.2 sec. b, Response to a feeble tug on a thread passed through the medial edge of the iris. Lead from a fine filament of the medial long ciliary nerve. Time, 0.2 sec. c, Same preparation and lead. Response to pressing on the anterior surface of the lens with a blunt glass rod.

Spontaneous discharge and increased intraocular pressure. Spontaneous activity in the long ciliary nerves was a usual feature of the preparations, especially when fresh and with the circulation in good condition. That the spontaneity originated in the eyeball and not in the nerve trunks, was established by severing the nerves at their emergence from the eyeball.

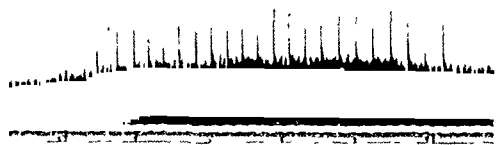


FIG. 4. Spontaneous discharge of large spikes in bursts, and of medium and small spikes irregularly. The contents of the eyeball were evacuated and the cornea excised. Lead from a fine filament of the medial long ciliary nerve. Time, 0.2 sec.

Examined in identifiable fibers, the most conspicuous spontaneous discharge was that already described as the "ticker." A second pattern is illustrated in Fig. 4; repetitive discharge within a period of about one second, of 18 to 24 large impulses, followed by a quiet interval of about 2 sec. The spikes in these discharges exceeded in size any obtained from the cornea, and the discharge persisted after the cornea was removed, and the iris, ciliary body, and lens were shelled out, with timing still as at the outset 3 hours before. However, the greater part of the spontaneous activity, especially that of small impulses, eluded analysis for pattern if indeed pattern existed. Examples of such activity may be seen in all the records.

Early in the course of this study it was discovered that when spontaneous activity reached confusing proportions, aspiration of fluid from the anterior chamber of the eye, or relieving the pressure by a small slit behind the sclero-corneal junction, materially reduced the activity, while injecting Ringers fluid or air into either anterior or posterior chamber intensified it or provoked it in quiet preparations. Figure 5 illustrates the sequence of raising and lowering intraocular pressure in an initially fairly quiet preparation. Although this observation has been most striking with the unresolvable spon-

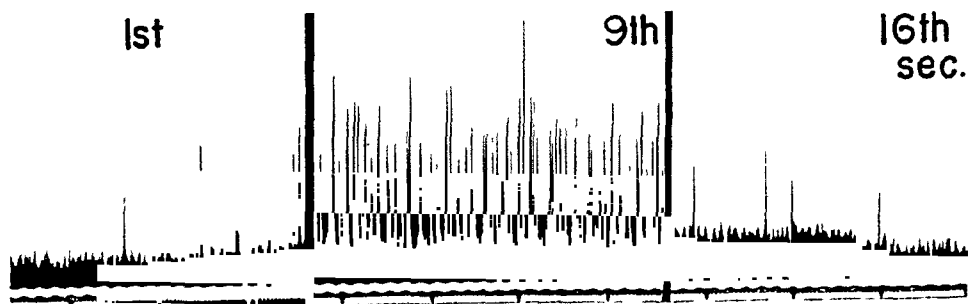


FIG. 5. Response to raising and lowering the intraocular pressure by injecting Ringer's fluid into the anterior chamber of the eye. Total duration of raised pressure, 15 sec. The hypodermic needle was inserted through the medial part of the cornea. Lead from the lateral long ciliary nerve. Time, 0.2 sec.

taneous activity of whole nerve leads, both the grouped discharges of very large impulses which were definitely not corneal in origin, and the evenly spaced ticker discharges of fibers of known corneal distribution shared in the effect. In the former, the number and frequency of impulses in a burst increased and the interval between bursts decreased as pressure mounted. The less conspicuous activity of the tickers was quickly submerged in the massive discharge excited as the pressure mounted, but if the pressure were then abruptly dropped so that that discharge rapidly subsided, the spontaneous activity of the ticker then required some seconds to reappear, a result which had otherwise been obtained only by strong stimulation in the fiber's corneal field. This inhibitory effect was most striking in one of the few fiber preparations, but the very fewness of the fibers remaining to innervate the cornea in this case, and the massiveness of the discharge during the period of raised pressure emphasized that increased intraocular pressure must excite afferent innervation which is largely located elsewhere in the eyeball than in the cornea.

SUMMARY

Arrangements were made for oscillographic recording of action potentials in the long ciliary nerves of cats in response to mechanical stimulation of the cornea. For lead, both whole nerves and nerves cut down until only a few fibers registered were used.

The neuron unit of afferent innervation thus isolated is a terminal ramification extending over a quadrant or more of the cornea and spreading onto the adjacent sclera and conjunctiva. This large terminal is spatially differentiated such that the lowest threshold and highest frequency of response are obtained in the central area, the one increasing and the other diminishing toward the periphery in all directions, while adaptation is most rapid both on the periphery and superficially. Within this large terminal adaptation and fatigue are both localized at the site of point stimulation, but some aspect of the activity, presumably the propagated impulse, spreads throughout to condition activity elsewhere. The total corneal sensory mechanism is an aggregate of such neuron units and not a continuum. Viewing the nerve-ending as all the terminal tissue of one nerve fiber, the properties of this receptor then appear as peripheral determinants of many of the qualities of the sensory experience derived from the cornea.

Some notes are included on afferent impulses excited by stimulating the sclera, iris and lens, and in response to increased intraocular pressure.

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LEARNING AND OTHER FUNCTIONS OF THE HIGHER NERVOUS CENTRES OF *SEPIA*¹

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INTRODUCTION

SURPRISINGLY little is known about the function of the higher nervous centres of invertebrates. Although much has been discovered about the capacity for learning and other more complex modes of behaviour especially among insects, yet there has been little attempt to reveal the activities within the central nervous system by which such reactions are mediated. This is especially regrettable because of the possibility offered by these centres of discovering something of the changes which occur during the process of learning, which in spite of so much study in vertebrates still remains almost wholly obscure (see Lashley, 1937). Yet in this subject, where great difficulties arise from the special and complex arrangements of the vertebrate nervous system, there is some expectation that comparative studies will yield fruitful results.

The central nervous system of the Cephalopods is especially well suited for such work. The supra-oesophageal centres are large, and there is reason to believe that the behaviour is complex and likely to include interesting types of learning. Moreover the animals lend themselves well to experiment, and the parts of the central nervous system are easily accessible for the purpose of operation.

Divisions of central nervous system

The main divisions of the central nervous system of the Dibranchiate Cephalopods are the paired optic lobes, and the supra- and sub-oesophageal ganglia (Fig. 1). However, each of these major regions is subdivided internally into lobes, which are the true structural and functional units. Some, though not all, of these lobes have been previously described and named (see especially Dietl, 1878; Thore, 1939), but details of their internal structure and fibre connections remain obscure, as does the part which each plays in the behaviour of the animal. For present purposes we may recognize centres of four types, as follows.

Lower motor lobes. The sub-oesophageal ganglia consist mainly of large neurons whose axons run, either directly, or after one further synapse, to

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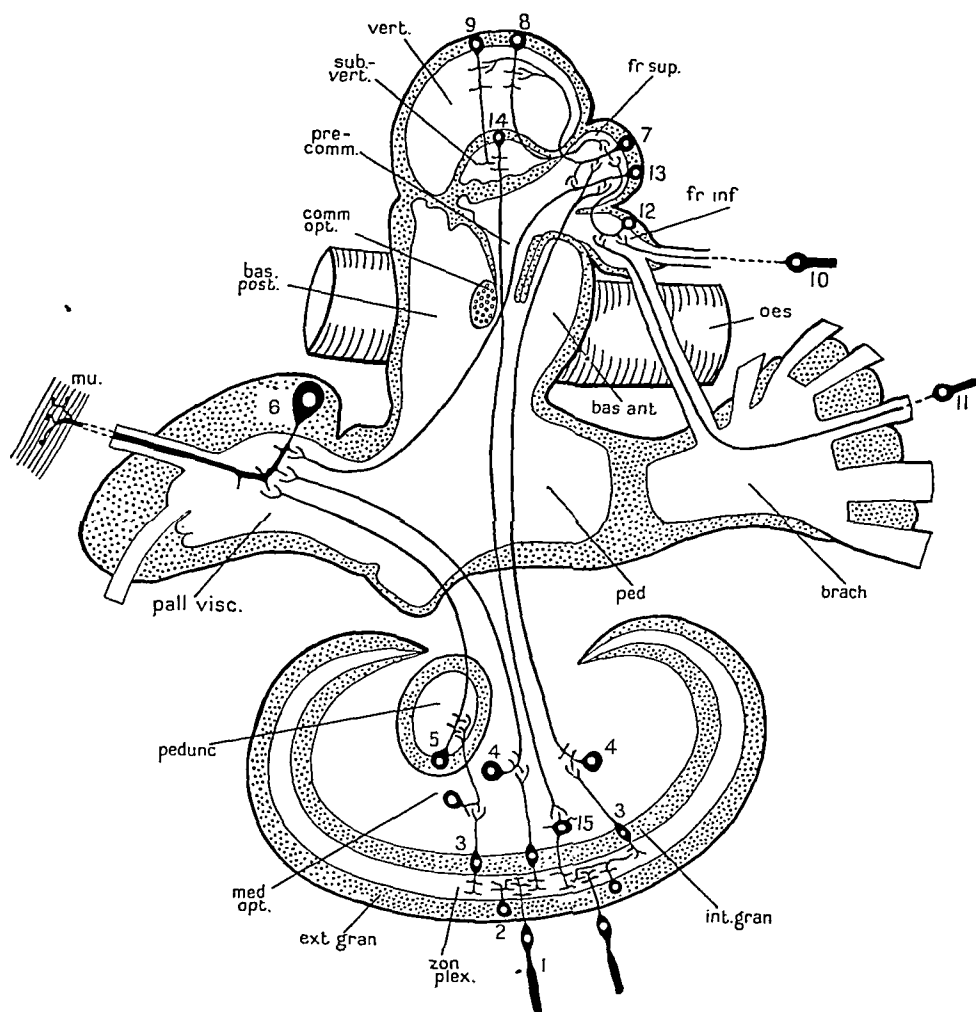


FIG. 1. Diagram to show some of the connections of the verticalis complex of *Sepia*, and its reciprocal relations with the optic lobes. The main outlines drawn from a single sagittal section, but the optic lobes much schematised.*

1, retinal cells; 2, external amacrine; 3, bipolar cells; 4, efferent cells of optic lobe; 5, cell of l. pedunculi; 6, final motoneuron; 7, cell of tractus frontalis superior-verticalis; 8, cell of tractus verticalis-frontalis superior; 9, cell of tractus verticalis-subverticalis; 10, visceral afferent; 11, tactile or chemoreceptory cell of arm; 12, cell of l. frontalis inferior; 13, cell of tractus frontalis superior-precommissuralis; 14, cell of tractus subverticalis-opticus; 15, centrifugal giant cell of internal granular layer.

(Legend continued on next page)

* The diagram is based mainly on the work of Cajal (1917) for the optic lobe and original data for the rest. The arrangement is certainly close to that shown but there is still some uncertainty about the following points: a) the exact form of the bipolar neurons 3; b) the connections of the efferent tracts of the lobus pedunculi 5; c) the relations of the sensory tracts 10 and 11 with the neurons 12, and of 7 with 13; d) the connections of the efferent tracts of the lobus frontalis superior 13.

muscles or other effectors (Fig. 1). Thus the arms and tentacles are controlled from the brachial and pedal ganglia, the funnel and eye-muscles from the pedal ganglia, and the mantle, fins and some of the viscera from the pallio-visceral ganglion. Electrical stimulation of these centres produces movements of the part innervated, and the movements are local, not synergic. In addition to the large motor neurons, these regions also contain smaller ones whose processes do not extend beyond the lobe itself. Sensory fibres from the skin also end at this level, and the centres are certainly able to act reflexly. After removal of one of these lower motor centres the part innervated is completely and permanently deprived of motor innervation.

The motor neurons of such regions may thus be considered comparable to the ventral horn and other parts of the somatic motor column of a vertebrate, and the lower motor centre as a whole, receiving afferent fibres and containing 'association' and motor neurons, is functionally not unlike the spinal cord.

Higher motor lobes. The more ventral part of the supra-oesophageal ganglia consists of lobes containing neurons of intermediate size, whose axons run to the lower motor centres. In this category are the lobus basalis anterior and posterior (Fig. 1 and 2), a hitherto unrecognised lobe the basalis lateralis, and the lobus pedunculi (= "ganglio del pedúnculo óptico" of Cajal, 1917), situated on the optic tract. All of these regions receive many fibres from the optic lobes and they may be considered as higher motor centres. Electrical stimulation of any of them causes movements of large groups of muscles, and after their unilateral removal circling and other forced movements of the animal take place, often in the form of continuous activity, as if their absence had freed the lower centres from an inhibition (Young, unpublished).

The influence of these lobes may therefore be compared in a general way with that of the regions of the mammalian brain which affect the 'tone' of

(Continued from preceding page)

bas ant, lobus basalis anterior
 bas post, lobus basalis posterior
 brach, ganglion brachiale
 comm opt, optic commissure
 ext gran, external granular layer of retina profunda
 fr inf, lobus frontalis inferior
 fr sup, lobus frontalis superior
 int gran, internal granular layer of retina profunda.
 med. opt, medulla of optic lobe
 mu, muscle
 oes, oesophagus
 pall-visc, palliovisceral ganglion
 ped, pedal ganglion
 pedunc, lobus pedunculi
 precomm, lobus precommissuralis.
 subvert, lobus subverticalis
 vert, lobus verticalis.
 zon plex, zona plexiformis of retina profunda

lower centres, for instance the red nucleus and other parts of the extrapyramidal motor system.

Primary sensory centres. These include the regions directly connected with the main afferent systems, and serving to elaborate the patterns of impulses sent from the periphery. The optic lobes, and especially their outer portions, the 'deep retina' (Fig. 1), are the most conspicuous formations to be placed in this section, but there are also olfactory lobes on the optic stalk (Young unpublished), and perhaps other regions which could be considered of the same nature. This type of centre is not always clearly marked off from

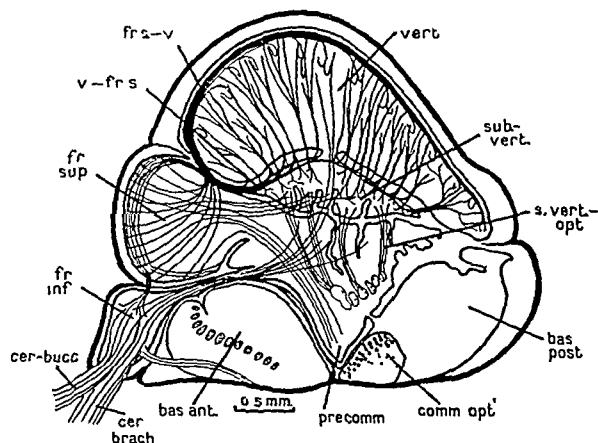


FIG. 2. Semi-diagrammatic view of median sagittal section of supra-oesophageal ganglia of *Sepia*. Outlines drawn from a single section, details schematic.

cer-brach.; cerebro-brachial connective.

cer-bucc., cerebro-buccal connective.

fr. s.-v.; tractus frontalis superior-verticalis.

s. vert-opt.; tractus subverticalis-opticus.

v.-fr. s.; tractus verticalis-frontalis superior.

Other lettering as in Fig. 1.

the previous one. The function of such a centre is to allow interaction between the impulses coming from the various parts of the receptor with which it is connected, so that reaction to shape or other qualities of stimulation may take place (see Young, 1938). Faradic stimulation of the centre of the optic lobe of *Sepia*, from which the processes of large cells pass to both supra- and sub-oesophageal regions (Fig. 1), produces definite movements, such as a general darkening or movements of the mantle or fins. But faradisation of the outer portion of the lobe usually produces no movements, presumably because so crude an agent as this stimulation is unable to simulate that patterned mode of activity by which this region normally controls the lower neurons.

Correlation centres. The highest centres, occupying the top of the supra-oesophageal ganglia, consist of very small cells, and are not directly connected with any single receptor or with the lower motor centres. These are the lobes with which the present work is mainly concerned, namely those

called by Dietl (1878) lobus frontalis superior and lobus verticalis, and the region below the latter which will here be called lobus subverticalis (see Fig 2) These three are intimately connected, and may be collectively called the *verticalis complex*

It was shown by Bert (1867) and von Uexkull (1895) that these regions are 'silent' areas, whose electrical stimulation produces no motor response This has been confirmed in *Sepia* during the present work Faradic stimulation of the lobus verticalis or frontalis superior with a monopolar electrode and a strength of shock considerably greater than that which produces responses from the lower centres produces no visible movements or changes of colour The results of such experiments must however be accepted with some reserve, since it is possible that the brief shocks produced by an inductorium are unsuitable for stimulating such small fibres

Study of the connections of the verticalis complex of ganglia throws further light on their function as 'higher centres' since it shows —(i) that they provide opportunity for the interaction of impulses from various receptor fields, (ii) that they have no direct connections with lower motor centres, (iii) that there are elaborate reciprocal connections between and within the lobes such as would allow of the presence of self re exciting chains of activity (see Young, 1938)

Connections of lobus frontalis superior The mechanism for the interaction of sensory impulses of various types is well seen in lobus frontalis superior (Fig 1 and 2) This is approximately of kidney shape, as seen in sagittal section, having a thick layer of cells over its anterior surface The axons of these cells run across the neuropil which occupies the centre of the lobe, and here they are crossed by layers of fibres from sensory sources, producing a characteristic criss cross appearance The tracts entering the lobe come from at least four sources, namely —(i) optic lobes, (ii) skin of arms and tentacles, (iii) receptors around the mouth and perhaps from the gut, by way of buccal ganglia, (iv) lobus verticalis (see below) The discharge of the fibres issuing from lobus frontalis superior is presumably controlled by the combined action of these various sets of fibres, though the exact manner of the interaction, and the significance of the positions of the various layers, remain very interesting subjects for future investigation

The fibres emerging from the 'hilus' at the hind end of lobus frontalis superior divide into three bundles passing to (i) lobus verticalis, (ii) lobus subverticalis, (iii) higher motor centres at the base of the supra oesophageal ganglia by way of an undescribed region to be called lobus precommisuralis (Fig 2)

Connections of lobus verticalis The opportunities for the formation of self-re exciting chains of activity are well illustrated by the connections of lobus verticalis (Fig 1 and 2) This has a thin outer layer and a very large central mass of neuropil The axons of the cells of the outer layer run downwards across the neuropil, giving off numerous axonic collaterals, and then collect into bundles which pass through into lobus subverticalis and either

end there or turn forwards to form the tract running to lobus frontalis superior which has already been mentioned. The main bundle of fibres entering lobus verticalis is that which arises in lobus frontalis superior. The two lobes can thus certainly influence one another reciprocally, and it is not unlikely that they can do so continuously, setting up a self-re-exciting system. The exact courses of tractus frontalis superior-verticalis and tractus verticalis-frontalis superior which complete the chains are shown in Fig. 3.

The existence of this type of circular connection was the starting point of the experiments to be described below, since it was suggested (Young, 1938) that possibly the cycle of activity is set up when impulses from various afferent sources interact, and that the maintenance of such cycles constitutes the

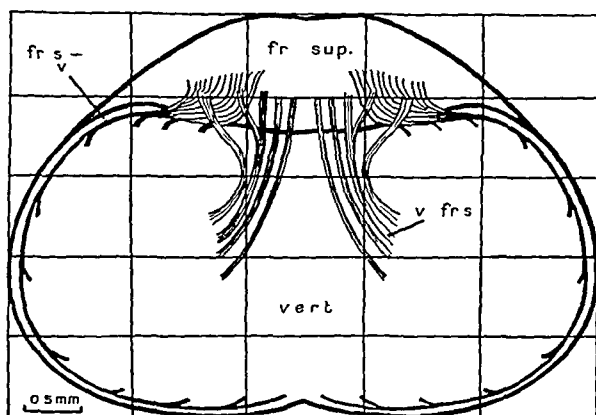


FIG. 3. Horizontal view of l. frontalis superior and l. verticalis of *Sepia*, showing the tracts connecting them with each other. For explanation of the co-ordinates see p. 503. Lettering as in Fig. 1 and 2.

basis of learning. It is not necessary here to recapitulate the details of the scheme originally proposed, but it may be noted that in addition to the cycle between lobus frontalis superior and lobus verticalis, the anatomical arrangements allow for a second and larger one, enclosing the first. For fibres from the lobus subverticalis run to the optic lobes, from which, as already explained, a large bundle passes to lobus frontalis superior. The activities of the verticalis complex can thus influence the discharges of those neurons of the optic lobes which run to the suboesophageal centres either direct or via lobus pedunculi.

The existence of these reciprocal connections must be of fundamental importance in the working of the centres, but it must not be forgotten that in the masses of neuropil lie many cells whose axons do not extend beyond the limits of the lobe in which they arise. The activities of such complex centres can hardly be wholly described in such simple terms as those employed above, which interpret all nervous function in terms of propagated nerve impulses. Yet perhaps no fundamental modification of the scheme pro-

posed would be necessary should we come to understand that masses of nervous tissue such as the neuropil of these lobes function by giving local rather than propagated responses when impulses are fired into them, such local responses serving to change the thresholds of the efferent fibres which issue from the mass.

Our purpose in the present work has been to make a first exploration of the functioning of these regions by the crude methods of stimulation and extirpation. In particular we have attempted to discover what effect on the behaviour, and especially on the powers of learning, is produced by the removal of lobus verticalis, or such part of it as would prevent its reciprocal excitation with lobus frontalis superior and stop the self-re-exciting chains described above.

The results so far obtained do not allow of a complete answer to the questions, but they show clearly that some at least of the more complex capacities of *Sepia* (such as that of following its prey out of the field of view) depend on the integrity of these highest supra-oesophageal centres.

Previous work on behaviour and learning in Cephalopods

In addition to the elaborate organisation of the central nervous system, the Cephalopods possess highly developed sensory and motor systems, and it is generally agreed that they show a complex or 'higher' pattern of behaviour. Unfortunately there has been very little attempt to justify this estimate by detailed observation or classification of the acts of which the animals are capable.

The 'higher' qualities of behaviour may be said to include:—(i) wide sensory and motor capacities, that is to say the ability to respond to varied aspects of the surrounding situation and to make this response in varied ways; (ii) the power to pursue an objective by indirect means, for instance to take a devious route in order to obtain food, or otherwise to show 'intelligence' in the solution of problems; (iii) the possession of conspicuous capacity for learning, i.e., allowing the functional state of the nervous system to change so that present response is modified by past experience.

The wide sensory and motor powers of the Cephalopods are well known and need no further comment. It is with the second and third of the above types of capacity that we are here concerned. There are very few reliable data about the ability of the Cephalopods to obtain their ends by indirect means. Pieron (1909) claimed that Octopods are able to uncork a bottle in order to obtain crabs seen through its glass wall, and similar statements are frequently made in semi-scientific literature. Unfortunately in no one case has sufficient detail or documentation been given to show whether the interpretations applied are correct. The observations detailed below show that *Sepia* is certainly able to follow a prawn which has moved out of its visual field, a by no means simple type of behaviour.

The data about the learning powers of these animals are slightly more satisfactory. Mikhailoff (1920) and Kühn (1930) have shown in Octopods

that formation of simple conditioned reflexes is possible, touch being the unconditioned stimulus used for conditioning coloured lights to produce chromatophore changes or flight reactions. Kühn was able to show that discrimination depending upon both wavelength and intensity is possible. Von Uexküll (1891) claimed that Octopods which have once attempted to eat crabs covered by Sea Anemones not only never do so again but actually refuse all further food. However, this remarkable conditioning is denied by Polimanti (1911).

The capacity to discriminate between visual shapes is highly developed, the eyes being well suited to the purpose (Alexandrowicz, 1927; Heidermanns, 1928). Tinbergen (1939) has recently shown, by studying the responses of *Sepia* to models of the opposite sex, that colour pattern as well as type of movement determines sexual recognition. It is highly probable, though no experiments have been made on the subject, that the prey is recognised by its visible shape.

K. and J. ten Cate (1938) have shown that *Octopus* is able to discriminate between a square and a triangle, or between large and small squares, though it is not clear how far size, rather than shape, was reacted to.

There is evidence, therefore, that changes may take place in the nervous system of Cephalopods which modify subsequent reactions. There appears to have been no previous work aimed at discovering the effects of removal of any part of the CNS on this capacity. Indeed there exist only fragmentary observations on the effect of operations on the CNS on the behaviour of these animals. Bert (1867) using *Sepia* and Fredericq (1878) with Octopods, claim that after removal of the entire supra-oesophageal ganglia the animals become wholly passive and show no further spontaneous movements. This was confirmed for Octopods by Phisalix (1892, 1894) who performed a number of operations on the CNS in connection with a search for the chromatophore centre. His observations were mostly concerned with chromatophore changes following operation, but he noted that superficial supraoesophageal lesions do not affect the movements of the animal, while injury to deeper parts produces considerable motor disturbances. Interpretation of all these observations is made difficult by the absence of histological control to show exactly what was removed. In the present work we have attempted to remove, or to interrupt the functions of, only those centres which, as we have seen above, would be expected to constitute the 'highest' portion of the CNS.

OPERATIVE TECHNIQUE

Operation on the supra oesophageal ganglia of *Sepia* is made difficult mainly by the profuse bleeding which follows opening of the 'cranium.' The animals are anaesthetised with 2 per cent urethane in which they become immobilized in a few minutes. Anaesthesia should be carried to the point at which all the chromatophores become contracted but the respiratory movements of the valves of the mantle still continue. This point is not easy to judge, and some animals were lost through carrying the anaesthesia to a point at which the respiratory movements stopped. Artificial respiration, either by pressing the mantle with the hand, or by direction of a stream of water over the gills is sometimes effective. The animals were wrapped in a damp cloth during the operation, which must be completed

in about fifteen minutes, unless a period of respiration followed by a second anaesthesia is given. No aseptic precautions were taken, but no serious infection was found.

A longitudinal incision is made in the skin between the eyes, and the 'cranium' exposed by severing the muscles attached to its anterior wall. A transverse cut is then made with a fine blade, low down through the front wall of the cranium, followed by longitudinal upward cuts such as to free a flap of cartilage which can be held up to expose the supraoesophageal ganglia. Profuse bleeding begins as soon as the cranium is pierced, and in spite of swabbing it is often necessary to operate in a pool of milky bluish blood. The animal may lose several millilitres, apparently without serious ill effects. The bleeding is due to the unavoidable rupture of the intra cranial venous sinus, but the arteries for the supraoesophageal ganglia and optic lobes run up at the sides of the *back* of the cranium, and are not interfered with by this anterior approach.

The superficial lobes of the supraoesophageal mass can, with practice, be distinguished externally, and incisions can be made into them as required with a fine sharp cornea knife. The lobes with which we are here concerned are the most superficial and can be easily removed. The whole of lobus verticalis can be sliced off without damage to any other centres. By entering lower down, lobus frontalis superior can be removed, with or without verticalis. Closure of the wound is effected by two or three stitches in the skin. In spite of the damage to the venous sinus post operative bleeding is very slight, and recovery and survival are good.

The effect of the operative procedure itself on the animal was controlled by making incisions into the skin and cartilage and then closing again without damage to the central nervous system. It is essential to control all lesions by histological examination, since their extent can never be properly determined at operation and autopsy, and secondary changes due to interference with blood supply must be looked for. Post mortem changes in the nervous system are very rapid, and all material was therefore taken immediately after the animals had been killed by decapitation. The eyes were removed, and the whole cranium and central nervous system then fixed in a mixture of 15 parts of 40 per cent neutral formaldehyde and 85 parts of sea water. Any attempt to remove the CNS from the head, or to explore the extent of the lesion at autopsy, leads only to uncertainty as to the extent of the true operative damage.

Material can be kept in formol for several months and is then stained with the modified Cajal's method described by Young (1939). The staining, however, should be undertaken as soon as possible, since with lapse of time the connective tissue is found to stain more strongly, and the nerve fibres less strongly. Good nerve stains can be obtained after one year in the fixative, but pieces kept for five years show only connective tissue.

All material was embedded in celloidin and sectioned serially at 50 or 100 μ . This procedure well repays the great labour involved, since it gives a very beautiful picture of the fibres in the neuropil and also often of their connections with the cells. Degenerating tracts can be identified during the first few days after operation by the rows of granules which they contain. Secondary pathological changes such as those produced by infection or anemia are easily recognised.

EXPERIMENTAL RESULTS

Removal of verticalis complex

Absence of motor defects or forced movements. Table 1 shows the extent of the injury, as revealed by serial sections, in the animals chosen for critical study. In order to map the lesion, the total number of sections (usually sagittal, occasionally transverse) occupied by the verticalis was counted, and divided by 5. The sections corresponding to each of 4 points across the hemisphere was then plotted on to a standard outline of the verticalis and frontalis superior. This outline was obtained from a suitable horizontal section, and is not exactly that seen from above, since the upper part of the verticalis overhangs the 1 frontalis superior. A standard outline of a sagittal section was then filled in to show the *maximum* depth of the lesion, which of course did not always extend as deeply as this over the whole area.

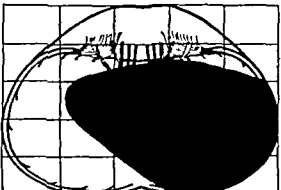

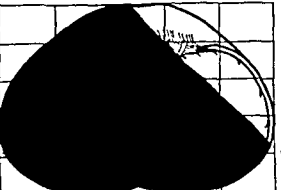

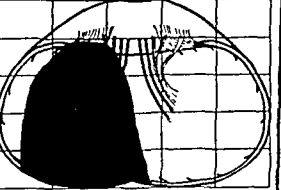

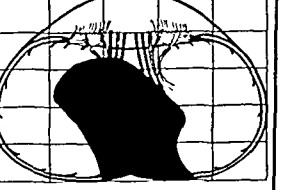

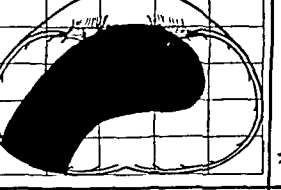

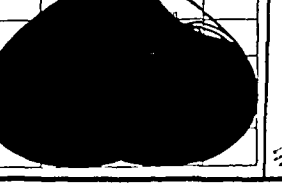



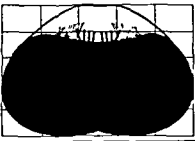

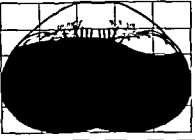

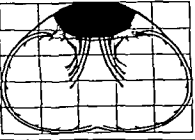

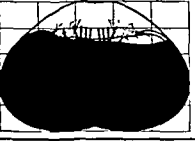



Sepia	Died or Killed	Survival, Days	Surface View of Lesion	Maximum Depth of Lesion
IJ	D	2		
IY	K	3		
LP	K	2		
NQ	K	3		
OA	K	17		
NU	K	2		

Table 1.—Diagrams of supraoesophageal ganglia of *Sepia* to show the

Sepia	Died or Killed	Survival Days	Surface View of Lesion	Maximum Depth of Lesion
PA	D	4		
PG	K	9		
PD	K	9		
PE	K	9		
PF	K	6		
PG	D	5		

extent of the lesions inflicted For method of compilation see p. 503.

Lesions of lobus verticalis and lobus frontalis superior do not produce any obvious effect on the appearance or behaviour of the animal. After complete removal of either or both lobus verticalis and lobus frontalis superior a *Sepia* swims normally with its funnel or fins, and its colour patterns (Holmes 1940) are unaffected in range or intensity. Moreover the animal can steer its way about the tank, avoiding obstacles, and darting away from an approaching stick without any symptoms of blindness, slowness, or any other disability. There are no clear signs of either increased activity (hyperexcitability) or passivity (depression or hypoexcitability). Without some measure of the activity of the animal such as is provided by cage-running in mice, it is difficult to be positive on such points, but the *Sepia* lend themselves very well to observation in a dimly lit glass-sided tank, and any gross disturbance in this respect would certainly be detectable. Indeed after certain operations on the more basal ganglia there is very marked hyperexcitability, the animal dashing recklessly about the tank after the slightest stimulus, or in other cases moving ceaselessly around: but no one of these symptoms is seen after the removal of the verticalis complex. In fact, *after such an operation the animal is quite indistinguishable in its simple behaviour from a normal Sepia.*

Moreover, after unilateral operations, such as those on animals LP and IJ, in which the verticalis was removed on one side only, *there are no forced movements or circling*, either with the fins or the funnel, though such movements are very pronounced after lesions affecting the more basal supraoesophageal centres, especially lobus basalis anterior (Young unpublished). Nor after such unilateral operation is there manifestation of asymmetrical colour patterns, though these again may be produced by deeper lesions.

Careful search for each of the above symptoms was made in every animal shown in Table 1. There is, therefore, every indication that lobus frontalis superior and lobus verticalis do not directly control any simple motor function, nor supply any tonic excitatory or inhibitory influence whose removal produces gross asymmetry in the animal.

Feeding after removal of verticalis complex. Still more surprising than the absence of motor defects after the removal of these centres is the fact that the animals are able to feed in a perfectly normal manner without them. A normal *Sepia*, when hungry and in the presence of a prawn, goes through a complex and well-marked series of reactions (see Holmes 1940). In the first position, which may be termed *attention*, the arms assume a characteristic posture, the two most dorsal being raised almost vertically and the sickle-shaped fifth pair being held out laterally. Meanwhile, waves of darkening pass over the head, and sometimes also over the body, the upraised tentacles being especially dark.

The next stage may be termed that of *approach*. The *Sepia* swims slowly forward towards the prey, following if the latter moves away. If the prawn moves out of sight at this stage the *Sepia* may follow it around a corner, a reaction which we term *hunting* (see below). As it comes nearer to its prey,

the 'tentacles' begin to protrude from the now forwardly pointing arms, and finally when the prawn is within range the reaction of *seizure* takes place, the long tentacles being shot out and the prey caught by the suckers of the expanded tip or 'hand.'

The whole set of movements is carried out in a 'purposeful' manner, each action being modified by circumstances and not following an invariable course. For instance, if the prawn is placed close to the *Sepia* in a clear space it will be rapidly seized, but if some other moving object is included in the field, or if the prawn is moribund or otherwise atypical, the 'attention' pe-

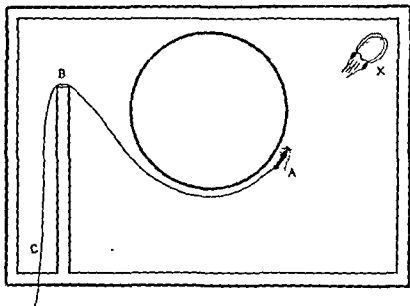


FIG. 4. Arrangement of tank to show capacity of *Sepia* to hunt when its prey passes out of sight, see text.

riod will be prolonged, and the approach very cautious, so that some minutes elapse before seizure is attempted.

After removal of *lobus frontalis superior* and *lobus verticalis*, a *Sepia* can attack and eat its prey in a perfectly normal manner, *provided that the food-object remains in the visual field until captured*. When a prawn was placed in the tank, 'attention', 'approach', and 'seizure' were seen to take place in all the animals in Table 1 in a manner indistinguishable from normal, providing that no 'hunting' was required. These centres are therefore not an essential part of the mechanism by which the shape of the food-object is recognised, or the hunger drive operated.

There is, however, some effect of the removal of these centres on feeding, since the animals often refused food for some days immediately after the operation, or, as in the case of animal PE, showed 'attention' but made no attempt at seizure of the food. No such changes were seen after a control procedure in which the cranium was opened but no damage inflicted on the CNS (see Fig. 5).

Inability to hunt after removal of verticalis complex. In order to investigate the capacity of *Sepia* to hunt food which disappears out of its sight, a black

thread was tied to the tail of the prawns, and the tank provided with a simple maze in the form of an enamel plate stretching three quarters of the way across the tank, and an enamel bucket (Fig. 4). The prawn was placed in the tank at A, in such a way that it could be seen by the *Sepia*, which was at rest in the corner at X.

If necessary the *Sepia* was steered to this point before each experiment, but this gave little trouble since nearly all animals chose this corner as a permanent point of rest during the day, always returning to it after excursion round the tank or capture of the prawn at the end of a trial. When the *Sepia* showed 'attention' and began to follow the prawn, the latter was withdrawn out of the visual field behind the bucket and allowed to rest at the corner B. If the *Sepia* followed round the bucket until it could again see the prawn, the latter was further withdrawn behind the enamel plate to C, where the *Sepia*, if it had followed round the corner, was allowed to seize and eat the prey. The presence of the black thread did not appear to interfere with 'attention' or feeding in any way.

The arrangement therefore makes it necessary for the *Sepia* twice to follow its prey after the latter has passed out of its sight, and the experiment thus tests the capacity of the animal to make a response to a situation in which no directly stimulating object is in the visual field. Put in another way it tests whether the animal is able to retain the association of a particular locality with the food-object which is no longer present there, and to use this association for its hunting. A great advantage of this experiment is that it investigates a type of response likely to be of use to the animal in its natural surroundings among the rocks, and is therefore perhaps of greater interest than discovery of the capacity of the animal to discriminate triangles from squares, or to learn the problems discussed later (p. 26).

When tested in this way, a normal *Sepia*, provided it is hungry and has become accustomed to the tank by living in it for a day or so, shows 'attention' as soon as the prawn appears, advances towards it, follows round the bucket and the edge of the plate, and eats it at C. The following is often rapid and the whole reaction accomplished within a minute or less. At other times the animal waits for as much as two minutes before gradually turning the corners. Occasionally, if the prawn is withdrawn very soon after having been placed at A, the *Sepia* does not follow round the bucket, but retreats again to its corner.

If the cuttlefish has been sated it usually gives no sign even of attention, much less of following, or it may give attention without following. The number of prawns which will be taken daily varies considerably; often it is only one, sometimes two or three. A hungry animal which has just finished a prawn will often immediately take another, perhaps even a third, but thereafter shows no further attention to prawns for some hours. If one prawn only is given daily, normal animals give the hunting reaction with complete regularity at each day's presentation.

After removal of lobus verticalis, however, the behaviour is very different. The *Sepia* shows attention to the prawn, and advances towards it, but

sight. The procedure on each of these days was to test the animal 4 times, twice in the morning and twice in the evening, by drawing the prawn away from it, so that each day's performance consists of four tests of the ability of the cuttlefish to hunt. On each day after the four tests, the *Sepia* was allowed to catch and eat a prawn. As will be seen from Table 1, subsequent sections showed that in this animal sufficient of lobus verticalis had been removed to interrupt tractus verticalis-frontalis superior on both sides, though large amounts of the lateral part of verticalis were intact, as was most of frontalis superior.

As will be seen from Fig. 5, essentially similar results were obtained with

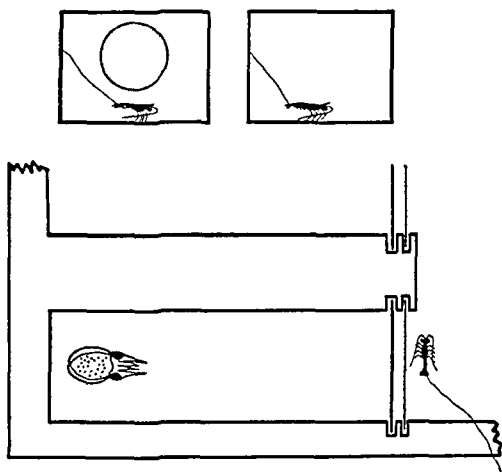


FIG. 6. Arrangement for testing reaction of *Sepia* to a prawn behind glass. Above is shown the end of the tank as it would appear with and without the glass and white spot.

animals NU, PA and PC when tested in the same way. Two further animals, PD and PE, were tested by a somewhat different, and as it proved less satisfactory, technique, using the tank designed for the learning experiments described in the next section. Each animal was confined in a separate runway (see Fig. 6) one end of which was closed by a sliding wooden door, which when opened revealed the prawn. As the *Sepia* moved up towards the door the prawn was removed sideways out of view, and a positive hunting reaction was recorded when the *Sepia* continued onwards through the doorway. The defect of the device was that the runway was too short, so that if the *Sepia* moved fast it was apt to be carried

through by its own momentum. Moreover it was difficult to withdraw the prawn fast enough to take it out of the view of the *Sepia* for more than a short time. In spite of this difficulty the results are included, since animal PD 10 times failed after operation to make the very simple hunting reaction necessary to capture its prey in this device. At three trials it did pass through the doorway, but in each of these cases it came up the runway very fast, and probably kept the prawn in view throughout.

Sepia PE was an animal previously tested and operated in connection with the learning experiments to be described in the next section. Accordingly no record was made of its ability to perform a hunting reaction before operation; however, every normal animal tested proved able to hunt. Tested in the runway *after* operation, it gave 2 successive trials in which attention and following of the moving prawn took place, but without the ability to perform the hunting reaction of coming through the door. These 2 animals,

therefore, provide further confirmation of the result shown by the other four, namely that following of food which has been moved out of sight is not possible after injury to the verticalis complex.

Experiments on learning in Sepia

A further series of experiments was designed to study the modification of behaviour as a result of experience, and the relation of this power to the verticalis complex.

Inhibition of 'seizure' reaction. It was noticed that if a glass jar containing a prawn was placed in a tank of *Sepia*, the animals at first attempted to seize the prawn, shooting their tentacles repeatedly against the glass. Gradually the attacks became less frequent, and finally the prawn was completely ignored. Clearly some change takes place in the nervous system such that a situation which at first elicits the attacks on the prawn later fails to do so. This inhibition of feeding reaction was further studied by keeping the animals in a tank provided with running seawater, divided by means of longitudinal partitions into a number of runways, each closed by a sliding wooden door (Fig. 6). One animal was confined in each runway for the duration of the experiment. The opening of the wooden door gave the animal access to an open space in the tank. In addition, slots were provided at the open end of each runway, so that a sheet of glass, as well as the wooden door, could be placed across the opening.

The animals were placed in the runways and allowed several hours in order to become used to the tank. At the beginning of an experiment the glass plate was placed in position across the end of the runway with a prawn behind it, the wooden door being kept closed. The door was then opened and the *Sepia* in the runway allowed to attempt to take the prawn, which it did repeatedly, shooting its tentacles violently against the glass plate.

It was found in the course of an experiment that this ejection of the tentacles became less frequent. The frequency of ejection was thus used as an index of the state of the animal, and, taking zero as the time of opening the door, the time (in seconds) at which each 'shot' was made was recorded. The experiment was ended when 3 minutes passed without any shot being made. The animal was then left undisturbed in the tank for a number of hours and then tested again with the same technique, the time of each shot being again taken until none had occurred in 3 min.

The animals were not fed throughout the experiment, which lasted at the most for ten days. They are able to starve for such long periods without showing obvious debility. In order to follow quantitatively the change which is occurring in the state of the nervous system as the learning proceeds, the number of shots per two minutes was plotted against time as shown in Fig. 7. With each of the animals tested it was found that the rate of tentacle ejection fell off from a very high value at the beginning of the first test period, until after 20-30 min. the criterion of no shots per 3 min. was reached. The curve is not smooth, but shows several minor peaks.

When the test was repeated after intervals up to eighteen hours, the animals usually made some shots at the glass, but *always less than in the first experiment*. The highest frequency of shots was often reached some minutes after the beginning of the test, and thereafter rapidly declined, the 3-minute criterion being reached much sooner than in the original test. Moreover the time taken to reach this criterion tended to become shorter at each successive test, the fall in this value showing a more or less regular 'learning curve.' Thus not only does the state of the nervous system change during the course of one test: the animal 'learns' to inhibit its seizure reaction: but also this

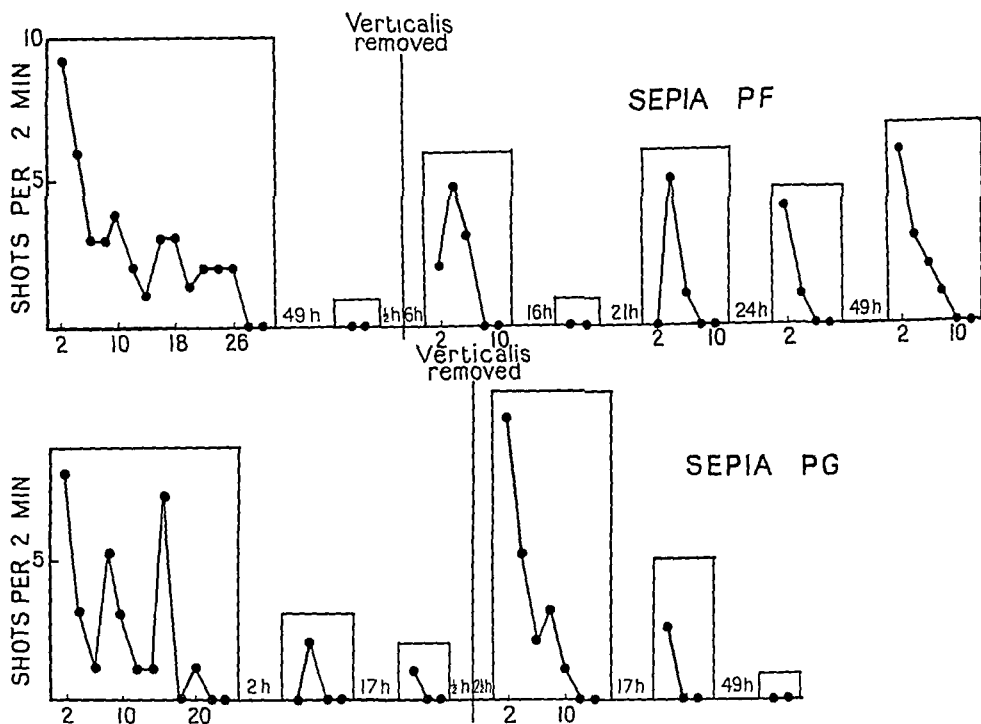


FIG. 7. Graphs showing the number of shots made at the glass per 2 min., before and after injury to the verticalis complex. The time scale for each test is in minutes and the periods between the tests are given in hours.

learning is carried over a period of at least 18 hours. But in most cases the change of state in the CNS, whatever it may be, which enforces this inhibition is partly reversed after such an interval. The animals have partly 'forgotten,' but quickly learn again.

Details of the maximum period of retention were not further investigated since the purpose of the experiment was to test the effect of the removal of the verticalis complex upon learning. The results are not wholly consistent. *Sepia PG* showed the clearest result. When tested after the operation it showed a higher frequency of tentacle ejection than at the very first test.

This is very remarkable since only 3 hours elapsed between this test and the last before operation, whereas the inhibition was not lost in the much longer periods between the pre operational tests. There can be no doubt that the operation has produced a marked effect on the learning. However, the experiment is uncontrolled since the effect of anaesthesia alone on the learning was not investigated. Much more significant is the fact that after this initial high value the ejection frequency fell off sharply. Therefore, even after an operation in which, as Table 1 shows, very nearly the whole of the lobus verticalis had been removed, the animal was able to learn to inhibit the seizure reaction. Moreover it learns to do so more rapidly than in the first test, that is to say some part of the changed state of the CNS is carried over in spite of the operation. It is therefore quite clear that the change of state involved in this particular act of learning does not occur only in the verticalis complex.

Sepia PF showed similar behaviour in that considerable traces of the

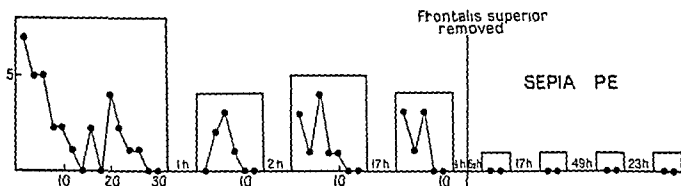


FIG 8 As for Fig 7. In this case the *Sepia* made no further shots at the glass after operation.

learning persisted over the operation, without, however any very high post-operative frequency of tentacle ejection.

Sepia PE gave a most curious result in that during five post-operational tests it at no time shot its tentacles at the glass. At each test the *Sepia* showed attention to the prawn throughout the experimental period, thrusting its head against the glass and moving excitedly up and down the tank, but never once were the tentacles ejected. In this case the operation, far from causing the animal to forget the learned inhibition, appeared to have made this inhibition more profound. It will be remembered that in the experiments detailed on p. 509, the feeding reactions were often affected during the period immediately after operation. But in these cases attention to the prawn was rare, whereas here there is clear evidence of attention, but a curiously accentuated inhibition of seizure. The operation performed in this case was the removal of lobus frontalis superior, not verticalis itself. It acts as a warning against treating all lesions of the verticalis complex as if they were equivalent, but in the absence of further evidence it is impossible at present to pursue further the questions which it raises.

In spite of the differences between the behaviour of these 3 animals, they

agree in showing that the change of state involved in learning to inhibit the ejection of the tentacles does not occur in the verticalis complex, since considerable traces of the learned state may persist after removal of this complex, and subsequent learning is at least as rapid as that before removal. The experiments indicate however that the verticalis complex may have some influence on the type of learning studied even though its presence is not necessary for the learning to take place.

Discrimination. The reaction of tentacle ejection was also used in a study of the ability of *Sepia* to learn a discrimination problem. The animals were presented with a prawn behind a glass plate marked with a white circle, and then learned not to eject the tentacles at this, while continuing normal ejection at a prawn presented without a glass plate. The discrimination was thus between 'prawn' as a food-object, and 'prawn plus white circle' as a non-food-object.

Apparatus and technique. The apparatus used in this experiment was a wooden tank divided into runways as in the case of the tank used in the study of tentacle ejection inhibition alone (Fig. 6). As the discrimination presents a much harder problem for the *Sepia* than the reactions previously described, and the experiment was consequently much longer, each runway was provided with a separate sea-water supply, and the animals kept permanently in this space for several weeks remained very healthy.

Every day each animal was given a group of 4 trials. These trials were of two types: (i) 'glass' trials in which the prawn was presented behind a glass plate bearing just above its centre a painted white circle two inches in diameter; (ii) 'feeding' trials in which the prawn was presented alone.

The technique of presentation of the prawn in the case of both types of trial was the same as that described for the experiments above. The daily group of trials was made up as follows: (i) Three successive 'glass' trials; (ii) The fourth and last trial of the day, a 'feeding' trial, in which no glass was used, and the *Sepia* allowed to reach and seize the prawn.

This grouping of trials was evolved to meet a practical difficulty, that of giving a number of trials on the same day. In the normal manner, an animal being trained in a discrimination problem would be given a number of trials of the two different types (e.g. in this case 'glass' and 'feeding' trials) arranged in a purely random order. However it was found in preliminary experiments that it was undesirable to feed the *Sepia* with more than one prawn a day. Thus if a 'feeding' trial preceded a 'glass' trial on any one day, the *Sepia* was unlikely to take any interest in the prawn at the second trial. A random arrangement of 'feeding' and 'glass' trials would thus limit the experiment to one trial per day. An alternate arrangement of 'glass' and 'feeding' trials was avoided, since it might possibly be objected that the animal was learning to respond to very alternate trial rather than to 'prawn' versus 'prawn+white circle'. As it is very unlikely that the CNS of *Sepia* would enable discrimination of every 4th trial from the rest, solely by virtue of its position in the series, the above arrangement was adopted. This gave the convenience of 4 trials per day, combined with the advantage of not feeding the animal until the last trial of the day.

In the case of 'glass' trials two indices of the state of the animal were taken, the time in seconds from the opening of the wooden door to the first

ejection of the tentacles, and the number of shots made in the first two minutes following this first shot. It was found that the time to the first shot lengthened and the number of shots made in the extra period declined during the course of the experiment. The discrimination was regarded as learnt when in each of the 6 consecutive 'glass' trials (*i.e.*, the trials of 2 successive days) no ejection was made in the first five minutes of experiment, while in the 2 accompanying 'feeding' trials positive responses (*i.e.*, feeding) were recorded. The number of shots made at the glass each day during the 2 min. is thus an index of the extent to which learning has taken place. When plotted against time the number of shots declines with the irregularity typical of most learning curves. When the total number of shots in three successive days of experiment are plotted, the general slopes of the curves appear, and are seen to be similar for the three animals investigated (Fig. 9).

Figure 10 is a diagram illustrating the progressive changes in the behaviour of the three animals during the course of this learning. At the beginning of the experiment 'glass' trials were invariably accompanied by shooting, but quite early, with all three animals, there appeared occasional trials in which there was attention or approach to the glass without any tentacle ejection. As the experiment proceeded, such trials, involving only attention or approach, grew more frequent, as did trials in which the *Sepia* ignored the prawn entirely, though continuing to seize the prawn in the accompanying 'feeding' trials. Finally, the criterion of learning adopted was reached. All 3 animals proved capable,

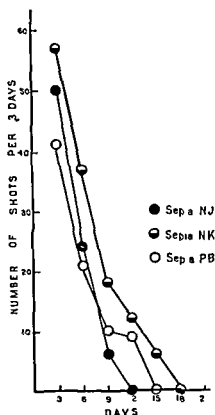


FIG. 9 Graph of course of learning not to shoot at prawn behind a glass plate on which there is a white circle. The ordinate shows the number of shots made at the glass (*i.e.*, 'mistakes') during successive periods of 3 days.

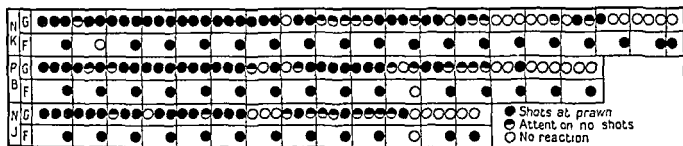


FIG. 10 Diagram to show the gradual failure of *Sepia* to respond to a prawn shown behind a glass plate provided with a white circle. For each animal the upper row of spots (G) shows the 'glass' trials and the lower row (F) the 'feeding' trials, there being three of the former and one of the latter daily. Vertical lines mark the days. During the experiment each animal gives progressively fewer trials at which there is attention or shooting if the white circle is showing, while continuing to shoot when it is absent.

therefore, of making a discrimination between 'prawn' and 'prawn+white circle' as objects of different significance. The criterion adopted was reached in the case of *Sepia* NJ after 38 'glass' trials, and in the cases of NK and PB, after 55 and 48 trials respectively. The discrimination is of a similar sort to that performed by the 2 *Octopus* of K. and J. ten Cate (1938).

A question arises concerning the importance of tactile influences in the setting up of this discrimination. In some of the trials in which there was attention and approach but no seizure, the animal 'felt' the glass with its tentacles, and of course, during the abortive shooting of the tentacles in 'glass' trials, the glass was struck violently by the ejected tentacles, so that tactile, possibly 'painful', stimulation must have been involved. Whatever tactual stimuli are involved in the learning, when the final criterion was reached, the *Sepia* remained immobile at the end of their tanks in the trials when 'prawn+white circle' was presented, a situation in which the *Sepia* has of course, no tactual relation with the glass plate.

The only further difference, other than optical, between the two situations in which positive and negative responses were given, is that in the 'glass' trials possible chemical influences emanating from the prawn are excluded. Although chemoreceptors are certainly present in Cephalopods, there is no evidence that they function as distance receptors. Indeed the fact that at the beginning of the experiment the *Sepia* does attack the prawn in 'glass' trials shows that the distance receptors involved are the eyes. Thus there is every reason to believe that *Sepia* can make a discrimination between the two visual situations.

Unfortunately the experiments were interrupted before we could make tests of the retention of this type of learning or of the effect upon it of the removal of the verticalis complex. However these experiments show for the first time that *Sepia* is capable of learning to make such a discrimination, and indeed that it lends itself well to this type of experiment.

DISCUSSION

From all of these experiments it is clear that changes of state may occur in the nervous system of *Sepia*, so that following certain situations the reaction capacities are altered. After a prawn has disappeared round a corner that corner exerts an attraction which it did not possess before. After a *Sepia* has repeatedly and vainly shot its tentacles at a prawn behind a glass plate, the futile reaction ceases to be given. After training, a *Sepia* will not attack a prawn if a white circle is also present, but only when the prawn alone appears.

Such modifications of behaviour are all examples of a process which may be called learning, and they show capacities which are likely to be extremely valuable to the animals in meeting their daily needs. This is especially clear in the case of the hunting reaction. A search for prawns among rocks would be difficult for a *Sepia* which was unable to follow its prey out of sight. The other situations studied, though they are never likely to be met with in the

life of the animal in this form, yet show capacities for modification by experience which could be applied to many situations.

These experiments, then, give us further knowledge about the behaviour and mode of life of the cuttlefish. But the main purpose of the investigation was to attempt to discover something of the nature of the change which takes place in the nervous system during the process of learning. All hypotheses hitherto suggested about this change have proved either inadequate to cover the facts or unacceptable to the physiologist (see Lashley, 1934, for summary). The particular hypothesis upon which the present work has been based was that the change taking place during learning consists in the setting-up of self-re-exciting activities which so facilitate the effects of impulses from sensory sources as to make them able to produce a response which they could not elicit before the training. Such a theory certainly allows us to understand some of the aspects of learning. For instance, once the activities have been set up, they might well affect large masses of nervous tissue, so that facilitation would be available for impulses from sources other than the peripheral receptors originally used in learning. Similarly, the degree or amount of learning, as expressed by the learning curve, would depend upon the number of neurons activated in a particular cyclical manner, and when large numbers are set in action at once, sudden drops in the learning curve, 'insight solutions' and the like are produced. Further, a possible basis is provided for the observation that the rate of learning depends on the quantity of tissue present (Lashley 1934 *etc.*).

In its present form, however, the theory does not give a clear view of the mechanism 'by which a response to a ratio of intensities is brought about' (Lashley, 1937). Thus during the present investigation it has been shown that there is some mechanism in the optic lobes by which a *Sepia* reacts to a prawn as a food-object, or by which the reaction of ejection is inhibited when the prawn is behind a glass plate. The element in these or any other discrimination reactions which is so difficult to understand is that they depend on giving a particular set of reactions to specific patterns of excitation received *anywhere* on the sensory surface. Thus we have to imagine that at least a large portion of the optic lobe is able to elicit a feeding reaction whenever the prawn shape is represented in it, even though individual elements are very variously stimulated at successive presentations.

The only contribution we can bring to this problem is the demonstration that discrimination of this type can certainly be performed in the optic lobes of *Sepia*, without the participation of the verticalis complex. After removal of the latter the cuttlefish not only still shows specific reactions to food-objects, but is able to reverse this discrimination, learning to inhibit the ejection of the tentacles at a prawn behind a glass plate.

However, it seems that the power to form the association involved in the hunting reaction is dependent on the integrity of the verticalis complex, and it is reasonable to suppose that this effect depends in some way on the re-activation of the optic lobes through the cyclical pathways shown in Fig. 1.

Thus it may be that the presence of the food object, in addition to eliciting the feeding reaction, also sets up processes in the verticalis complex which so influence the optic lobes as to cause associated configurations, such as the corner round which the prawn has disappeared to elicit following reactions which they alone could not produce. Since we know nothing of the method by which one configuration rather than another produces its effect it is not profitable at present to speculate further on this baffling theme.

The hypothesis of self-re-exciting chains, in the simple form of the interaction of lobes considered above, is clearly inadequate to meet all the requirements of learning situations even as simple as those here studied, which demand a knowledge of how masses of tissue, such as the optic lobes, change their state so as to become responsive to certain configurations projected upon them. It is by no means excluded that self-re-exciting activities within the lobes play a central part in such changes. For further advance we require more information about the structure and mode of activity of the centres in which such changes take place. There is everything to be gained from study of these centres in as many animal types as possible.

The present study has at least shown something of the capacities of *Sepia* and of the activities of its higher nervous centres. Perhaps the most outstanding point is that removal of the verticalis complex not only does not produce any gross motor abnormality but does not even prevent discrimination, or feeding, though it may affect these processes indirectly, and certainly makes the hunting reaction impossible. The optic lobes alone, as we have come to realise ever more fully as the investigation proceeded, are able, with their relatively uniform, if complex, structure to mediate by themselves many of the more elaborate types of behaviour.

SUMMARY

1. Four types of centre are recognised in the CNS of *Sepia*, (a) *lower motor centres*, in the sub-oesophageal ganglia, comparable to the spinal cord of mammals; (b) *higher motor centres* at the base of the supra-oesophageal ganglia; (c) *primary sensory centres*, such as the optic lobes, and (d) *correlation centres*, occupying the dorsal region of the supra-oesophageal ganglia.

2. The correlation centres, the verticalis complex, allow opportunity for interaction between impulses from various afferent sources, and send tracts to the higher motor centres and to the optic lobes.

3. Faradic stimulation of the lobes of the verticalis complex produces no visible movements; they are 'silent areas.'

4. After complete removal of the verticalis complex the behaviour of a *Sepia* shows no superficial abnormalities. The animal is not blind and can steer and move around as if normal. There is no evidence of either hyper-excitability or depression.

5. After unilateral removal of these centres there are no forced or circling movements, or colour asymmetries, though such are found after injuries affecting the more basal centres of the supra-oesophageal ganglia.

6. After complete removal of the verticalis complex a *Sepia* shows the reaction of attention to a prawn and advances towards it, seizes it and eats it in a manner indistinguishable from normal.

7. A normal *Sepia* is able to hunt, that is to continue to follow a prawn which passes out of its sight round a corner.

8. After removal of the verticalis complex *Sepia* is unable to hunt in this manner, and can then catch its prey only if the latter remains within the visual field.

9. If a normal *Sepia* is presented with a prawn behind a sheet of glass it will shoot its tentacles at the glass, but with a frequency which steadily diminishes. When tested after intervals as long as eighteen hours, considerable traces of this changed state are still evident, the tentacles not being shot as frequently as in the first test, and complete inhibition being more rapidly reached.

10. Removal of the verticalis complex disturbs this state of learned inhibition in various ways, but distinct traces of a previously learned inhibition are seen after removal, and relearning continues to be at least as rapid as the initial learning. The change of state concerned in this learned inhibition does not therefore necessarily involve the presence of the verticalis complex.

11. Three *Sepia* were trained not to eject the tentacles at a prawn showing behind a glass plate on which a white circle was painted, while continuing to shoot at prawns not accompanied by glass and circle.

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CONVERSION OF PHASIC INTO TONIC MOVEMENTS BY PYRAMID LESIONS¹

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INTRODUCTION

IN A PREVIOUS REPORT (Smith, Mettler and Culler, 1940) evidence was presented tending to show that the phasic response which is characteristically obtained by cortical stimulation depends, for its clonic factor, upon the integrity of the kinesthetic pathway of the region involved. It was further indicated that, while the primary locus at which the kinesthetic impulses influence the descending flow is at the spinal level, another pathway in the region of the ventral spinocerebellar tract is to some extent involved.

The importance of an investigation into the character and factors concerned in the production of a cortically induced movement need not be here emphasized but it should be pointed out that our particular interest in such movements resulted from the observation that they are altered by extrapyramidal stimulation (Mettler, Ades, Lipman and Culler, 1939). Such movements may be inhibited through the caudate, "held" by pallidal stimulation and also influenced by subthalamic and nigral stimulation. If the movement under investigation, however, is itself a complex it is desirable to resolve it into the simplest elements possible. The present investigation was designed to (i) check the previous results reported by Smith, Mettler and Culler and (ii) discover if the cortically induced phasic movement could be converted into a tonic movement by any procedure other than those previously reported.

PROCEDURE

In our earlier experiments "adult cats were anaesthetized with ether and the cerebral cortex of both hemispheres exposed. The animals were supported in a horizontal position with the legs hanging freely. The motor area was stimulated by using an inductorium and a bipolar silver electrode (2 mm separation). The stimulus was adjusted until a continuous rise to a phasic response" This procedure, excepting where different circumstances are noted and with the exception that a half-rectified 60-cycle stimulator was employed in place of the inductorium in all cases but those specifically noted below

EXPERIMENTAL DATA

1. Extension of previous results

In our previous experiments when the dorsal roots from and including C5-Th1 were cut cortical stimulation invariably produced tonic instead of phasic movements

a. *Chronic deafferentation* A possible objection to this result may be made because the experiments reported were of an acute rather than chronic nature. In spite of the fact that we previously presented reasons for considering cord trauma a negligible factor, 7

¹ Financed by the Milbank Memorial Fund.

animals were prepared under sterile precautions and the dorsal roots from and including C5-Th1 were cut by the intradural route on one side. Each of these animals was examined one day later. Three did not move the deafferented limbs in spontaneous struggling and in righting themselves, four did. When the cortex was exposed, the three former were paralytic in the deafferented leg while the four latter responded by tonic flexion of this limb, no phasic movements being elicitable.

It is possible that the intradural route may be considered an objectionable one. In order to obviate any possibility of intradural damage four cats were now operated extradurally. The left dorsal roots of nerves C5-Th1 inclusive were exposed, under sterile precautions, in the corresponding intervertebral foramina. After 3 days the cortex of one of the animals was exposed bilaterally and stimulated. Movements of the right forelimb were phasic as usual but only tonic movements could be elicited from the left forelimb. The cortical point having the lowest threshold for this response was the lateral portion of the anterior sigmoid gyrus. The remaining 3 cats were tested on the 4th, 5th and 6th days respectively. Only tonic responses could be evoked from the deafferented limbs. No animals were allowed to run longer than this time since it was concluded that little would be gained by a longer postoperative interval and that regeneration might complicate the results. None of the animals showed any infection and all were moving about on the 3rd day. Examination of the 3 animals allowed to run 4 days or longer showed, at that time, that while they were anesthetic in the limb concerned they could still move it. In walking about (upon the 3 good legs) the anesthetic limb was phasically waved in a semiflexed position. The movements corresponded in a general way with the locomotor pattern. There was little evidence of strength in these vague gesturings. Clonic activity was also manifested in the deafferented limbs during deliberately provoked epileptic seizures and, after the opposite leg had also been deafferented (intradurally), epileptic seizures evoked from the forelimb area still spread to the hind limbs of the animals.

b. Devascularization. In cutting the dorsal roots, by any approach, there is always a certain amount of interference with the vascular supply of the cord. Usually one or two large vessels lying in association with the dorsal root fibers at one specific level are more significant than any or all of the others. By the intradural route such significant vessels may be identified and avoided. We did not therefore consider vascular disturbance to be a significant factor especially since a single lesion applied to the side of the cord, in the region of the ventral spinocerebellar tract, and which involved no widespread vascular disturbance could also convert a phasic into a tonic movement. More positive proof is, however, needed before it may be concluded that interference with the vessels accompanying the dorsal roots is not the agent responsible for the results here reported.

In order to test the vascular factor five animals were prepared by exposure of the cord segments concerned and the dorsal roots from and including C5-Th1 were cut on the left. An ultropak with a light green filter was now swung over the right side of the cord and the vessels of the dorsal roots of C5-Th1 were separated from the nerve filaments and severed. The first animal was found to be paralysed after this procedure. In the second and fifth the movements, after a preliminary period in which only tonic responses occurred, became phasic but it was found, after despatching the animals, that a few small vessels had escaped section. In the third and fourth animals no intact vessels could be found travelling over the dorsal roots concerned.

Immediately after cutting the vessels, stimulation of the left cortex of both of these animals gave tonic and not phasic responses. After the lapse of some minutes phasic movements were elicitable. We cannot find any figures in our records on the precise length of time the tonic activity persisted but our recollection is that it was in the neighborhood of five minutes. This would seem to imply that the tonic stage was not so much the result of anemia as of excessive handling of the dorsal roots and mechanical damage (suction had to be repeatedly used to clear the operative field). The point has, however, no direct bearing upon the main issue which is that phasic activity is elicitable after the vessels entering over the dorsal roots of C5-Th1 inclusive have been severed bilaterally, a much more serious vascular interference than was present in any of our regular experimental animals.

c. Section of spinocerebellar tracts in chronic animal. Following our initial communication in which we reported abolition of phasic activity by lesion in the region of the ventral spinocerebellar tract the question was repeatedly

raised as to whether or not the effect might not be due to interference with a descending instead of ascending tract. To this disturbing criticism we opposed the following line of reasoning: (i) abolition of incoming sensory impulses is sufficient to convert a phasic into a tonic movement and it is unreasonable to assume a separate mechanism to be responsible in cases in which the lesion is more centrally placed; (ii) histologically verified lateral column lesions when not apparently encroaching upon deep tracts were sufficient to convert phasic into tonic activity and (iii) when a lesion could be found which obviously encroached upon deeper tracts it always came from an animal which had been paralysed by the lesion. We were finally forced to admit that this evidence was only of a presumptive nature and set about to gather more conclusive data.

Our first efforts were directed toward an attempt to cut or fulgurate the ventral spinocerebellar tract at the level of the decussation of the pyramids in order to eliminate one of the two main descending tracts from consideration. We were never able to devise a technique which was satisfactory for the approach. All animals in which the spinocerebellar tracts were cut succumbed before they could be satisfactorily stimulated. In some cases they could be kept alive by artificial aids but the cortex was thoroughly unreliable.

Attempts to solve the question by Marchi study also failed to give reliable results. In order to place a cord lesion correctly it is necessary to expose the cortex for stimulation so that the conversion from a phasic to a tonic response can be verified. Such a procedure cannot be carried out without obtaining a certain amount of pyramidal degeneration originating in the motor cortex and thus the purpose of the experiment is vitiated. Besides the cord itself undergoes extension of the original lesion and fibrosis which may produce paralysis and a variety of complications difficult to evaluate. It became apparent that the problem would have to be approached by an entirely different technique.

2. Other procedures designed to convert a cortically induced phasic into a tonic movement

a. *Cerebellar ablation, section of the brachium conjunctivum and lesions of the cerebellar nuclei.* If the conversion of a phasic into a tonic movement be truly the result of spinocerebellar section then, presumably, removal of the cerebellum might be expected to produce a similar result.

In accordance with this hypothesis 7 cats and 1 dog were subjected to complete cerebellar ablation. By the use of the ligature and suction technique checked by intracranial illumination this is a relatively simple operation and can be rapidly performed with very good results. None of these animals gave any evidence of tonic conversion. Upon cortical stimulation all responded with loose, flabby, rapid, phasic forelimb movement.

It might be objected that total removal of the cerebellum is not a reliable test of the original hypothesis because it is perfectly conceivable that an internal imbalance in the cerebellum may be the factor responsible for converting a phasic into a tonic movement. Were this so removal of the cerebellum from an animal in which the phasic response had already been converted into a tonic one, should reestablish phasic activity. This, however, does not occur.

One cat was prepared by dorsal root section and six by lesion in the region of the ventral spinocerebellar tract at the 4th cervical segment. In each case the movements continued tonic after cerebellar ablation. In 4 of the last 6 cases the movements were

notably changed in character (following cerebellar ablation) in that there was a distinctly tremorous element in the tonic movement and it was not so well nor so long sustained as previously. Neither were the movements of the cats in which the spinocerebellar region had been previously fulgurated of the loose type usually seen following simple cerebellar ablation alone.

It remains to be seen what results are obtained by smaller more selective lesions of the cerebellum and what can be gained by fulgurating the cerebellar nuclei or cutting the two upper brachia. In the following experiments the lesions of the brachium pontis were made by a direct visual approach through the basioccipital and basisphenoid following resection of the larynx, esophagus and prevertebral muscles. Although this sounds like a formidable operation it is really quite simple¹ and almost completely bloodless if done with reasonable care. The removal of cerebellar cortex was also done by the direct visual approach (dorsal, of course) while the deep lesions were made by means of a unipolar tungsten electrode carried in the cerebellar attachment of a Horsley-Clarke instrument.

In 2 animals the brachium conjunctivum was fulgurated on one side. This did not abolish phasic movements on either side. In another, the decussation of the brachia conjunctiva was thoroughly fulgurated. Following this procedure the animal developed a position of antigravity rigidity. In spite of the extreme extensor hypertonia of the forelimbs cortical stimulation of either side still evoked phasic movements. It was, however, noticed that stimulation of the medial side of the anterior sigmoid gyri was more effective than that of the lateral side. Another animal which showed such antigravity rigidity had a more orally placed lesion which fulgurated the decussation of the rubrospinal tracts and, in part, also the red nuclei themselves. Here, too, phasic movements were elicitable but the foreleg was never brought farther forward than a vertical line dropped through the scapulo-humeral joint. Our notes on this case state that "phasic movements of the . . . leg seem to illustrate that it is impossible for the flexors to be operated. In other words the limb seems to be habitually held in a position in which the muscles on the anterior side of the leg are all paralytic and those on the posterior side are tonically contracted. When the cortex is stimulated these extensors seem to be capable of contraction and relaxation [in the production of a phasic movement which occurs entirely posterior to the line of the vertical position]. If the limb is deliberately placed in front of this vertical position it becomes extremely tonic but behind this position it is rather loose."

Removal of the anterior (1 cat) and posterior halves (1 cat), either lateral lobe (2 cats and one dog) or of the vermis and roof nuclei (1 cat) of the cerebellum did not convert phasic into tonic movements. It may be worth noting that, in the latter case, the forelimb movements were decidedly hypertonic. When the lateral lobes were subsequently removed from this cat a typical loose, acerebellar, phasic movement resulted. In another animal in which the globose and emboliform nuclei were both fulgurated, on the right side, there was produced flexor hypertonia on the left and extensor hypertonia on the right. The left leg also developed an athetoid quality. Phasic movements were still elicitable from both cerebral cortices. Fulguration of both nuclei fastigii in another animal produced no such result and did not convert phasic into tonic movements nor did fulguration of the nucleus dentatus in still another animal. In several other animals in which perfect "hits" were not obtained but in which the fulguration approximated the above situations similar results were observed (no effect from the region of the nuclei fastigii and dentatus but motor deformation from the region of the globose and emboliform nuclei.)

The supposition that the inhibitory effect of the cortex is mediated through the cerebropontine fibers and exerted upon the cerebellum has often been advanced. Evidence both in favor and against this theory has appeared. While it would seem unlikely that this system could have been involved in our original experiments it was decided to place a series of lesions

in the brachium pontis to investigate the possibility of conversion of phasic movements into tonic activity by this means.

The pons was accordingly, in one cat, severed in the midline. Following this procedure stimulation of either cortex still gave good phasic movements which presented little difference from those usually obtained except that, as the leg reached the position of full extension, a peculiar spring-like quality was noticed in it. The animal showed a rather greater degree of spontaneous activity than is usual but this was of an orderly and slow type and manifested itself in the appearance of a pattern of "walking." There may have been some accentuation of extensor hypertonia and a rather well-marked "magnet" reaction appeared in both forelegs.

Since unanimity of opinion upon the total crossing of the pontine fibers has not been reached and since it is possible that some opposition between the two sides of the middle brachium exists it was decided to sever the lateral side of the pons. None of these attempts were completely satisfactory, some involved extraneous systems and none were complete. It was subsequently found that the pons could be eliminated as a responsible factor by another process so this method of approach was discontinued. In no case did we obtain tonic movements from these experiments; in many cases the movements which were obtained were very tremorous though not of a typical acerebellar type and in many the threshold of cortical excitability was found to be remarkably reduced after unilateral section of the pons arm. After-discharge was frequently encountered.

While the exact significance of some of the above results is not entirely clear it seems certain that there is little evidence in favor of our original assumption that spinocerebellar tract injury is the responsible factor in the conversion of cortically induced phasic movements to those of tonic type. We may also question the importance of rubrospinal injury in this connection. The problem now arises as to whether, if the brain be entirely disconnected from the cord with the exception of the corticospinal tracts, phasic movement is possible.

b. The role of the corticospinal tract. In order to answer the question just raised the following experiments were performed.

In one cat a ligature was dropped in front of the tentorium in such a way as to disconnect all the neural tissue dorsal to the cerebral peduncles. A certain amount of antigravity rigidity developed but phasic movements were elicitable as usual. Since in this case the corticopontine and possibly corticorubral fibers may have still been operative we prepared a second experiment in which we were successful in producing one animal. In this case the cerebellum and quadrigeminal plate were ablated. The tissue dorsal to and between the pyramids, in the region of the mesencephalon, was now removed. This included the red nuclei. The right peduncle was injured but stimulation of the left cortex still gave phasic movements in the right foreleg. There was still, in this case, considerable tissue left in the pontine region which might conceivably have been eliminated. In order to get rid of this a third experiment was devised in which we were able to keep one cat alive and in good self-sustaining condition. The pyramids were exposed by the ventral approach and a ligature was placed dorsal to them entering the medulla at the lateral edge of one and emerging at the lateral edge of the other. A hole was now made in the occipital bone and the cerebellum was removed. The free ends of the ligatures were next brought about the respective sides of the medulla and over the 4th ventricle. A knot was now tied in these. The result was a practically complete separation of the medulla from the pons with the exception of the pyramids. Microscopic examination, after disposal of the animal, showed, on the right

side, all of the medulla to be cut through excepting the right pyramid and an adjacent portion of the trapezoid body and medial lemniscus in the midline. On the left, a small amount of tissue lateral to the pyramid (region of the spinothalamic tract) in addition to the trapezoid tissue had also escaped. The only element of hypertonia noticeable was a very slight resistance to passive flexion of the right forelimb and a somewhat more marked resistance in both hindlimbs, more especially of that of the right side. Stimulation of either cortex produced phasic movements. These were loose and tremorous and had a low threshold. Stimulation of the right cortex sometimes caused slow elevation of the tail which was then temporarily held in this position. The medial sides of both anterior sigmoid gyri were more easily excited than the lateral portions. It may be interesting to note that we were unable to induce any epileptic seizures in this animal despite the application of extremely high current densities to the cortex.

We may conclude then that there is no element which lies oral to the medulla which does not travel by way of the pyramids and which is necessary for the phasic factor in cortically induced movement. The corticopontal fibers are among the elements thus eliminated. It remains to be seen what factors within the pyramids themselves may be involved but before we cite the results obtained by pyramid section let us examine what forelimb movements are elicitable from the cerebral cortex and how these representative points are arranged. Many maps of the excitable cortex of the cat have been prepared and published. Most of these show very poor agreement. The cause of much of the disparity seen is probably to be sought in the different techniques employed during stimulation and in the difference in directing interest. A large number of diverse results may in fact be easily obtained from the anterior sigmoid gyrus, especially by specifically denervating extraneous muscle groups, but with regard to forelimb movements in the intact animal only there is an underlying fundamental pattern. This pattern may be expressed briefly by saying that flexion (caudal) of the humerus upon the scapulo-humeral joint is dominated by the posterior half of the anterior sigmoid gyrus, extension of this bone by the anterior half, extension of the forepaw by the cortex lateral to the cruciate sulcus, abduction of the limb by the lateral half of the posterior sigmoid while flexion and extension of the elbow lie between the scapulo-humeral and forepaw areas.

In the intact (neurologically), mature cat a very small unipolar electrode is necessary to bring out this pattern. We have usually employed a silver spring of 0.25 mm. diameter wire for the active electrode (held in place by a special attachment of the Horsley-Clarke instrument) and the frame of the Horsley-Clarke itself for the indifferent electrode. With a variable-frequency stimulator it is possible to get fine discriminating and tonic movements from the optimal portions of these representative points. If the electrode is placed at neutral, intermediate points (the regions which the older English experimenters formerly called "zones of confusion") phasic movements occur and if a unipolar ball (2 mm. diameter) or bipolar electrode is used nothing but phasic movements result if the cat is in good condition. If there is hemorrhage from the pial vessels, stasis of the superior longitudinal sinus, damage to the cerebral tissue, general "shock" or, if the cat is young, tonic movements are likely to be the only variety elicitable. (In the following

experiments the bipolar, wandering electrode was used on the cortex as usual.)

The pyramids were exposed by the ventral approach in one animal and a lesion of the ventral surface of the right pyramid was made with a small cautery tip. The cortices were exposed and stimulated. Stimulation of the left cortex gave phasic movements as usual but stimulation of the right produced tonic movements only. In this animal it appeared to us that there were similar movements in the tonic (left) manner and more attenuated. Excitation of the lateral portion of the anterior sigmoid gyrus produced tonic flexion but tonic extension was obtained from the medial portion.

Ten animals were now prepared with unilateral subtotal pyramidal lesions of varying size and position, in the right pyramid, just below the pons. The ligature instead of cautery was used. No correlation between the physiologic result and position of these lesions could be established upon microscopic examination. It was, however, observed that very small lesions regardless of position were accompanied by the abolition of the phasic factor and the appearance of the dissociation of flexion and extension noted above. In the case of large lesions the medial portion of the gyrus was inexcitable and tonic extension could not be evoked but no matter how large the lesion was (nor apparently no matter how it was placed, *i.e.*, whether laterally or medially in the pyramid) tonic flexion could always be evoked if any pyramidal fibers were left intact. Thus a situation in which tonic extension alone could be produced was never obtained. Extirpation of the opposite cortex (from that being stimulated) and isolation of the motor cortex of the same side from its surrounding parietal tissue never affected the nature of the above results except insofar as they caused deterioration of the preparation.

That these effects are mediated by the pyramids and nothing else is strongly indicated by various results which have been reported above but it is a simple matter to prove conclusively that this is the case by complete section of one pyramid alone. If this is done carefully, stimulation of the opposite cortex gives perfect phasic movements while stimulation of the anterior sigmoid gyrus of the same side produces a generalized inhibition of movement. This experiment was performed on two cats. Inhibition by cortical stimulation following double pyramid section has already been reported by Tower and Hines (1935). Our results are in complete accord with their report and add the insignificant information obtained by cutting one pyramid alone and at a different level from the one they chose. Epileptic seizures could easily be induced in all animals of the above type by stimulating the cortex corresponding to the cut pyramid (opposite to it) whereas, it will be recalled, they could not be induced when only the pyramids connected the cortex with the cord.

None of the above animals in which the pyramids were traumatized exhibited any evidence of spasticity nor hypertonia in such spontaneous movements as they displayed in the few short hours during which they were kept. These movements moreover showed the usual phasic elements.

DISCUSSION

The question now arises as to how partial section of the corticospinal tract can bring about the above noted conversion. From this and the earlier, report we have the following observations to consider: (i) deafferentation abolishes the phasic factor in cortically induced movement; (ii) so also does corticospinal tract damage; (iii) flexion and extension may be dissociated in the cortex by pyramid damage; (iv) extension can be completely eliminated by partial corticospinal section but flexion cannot; (v) discriminating cortical stimulation gives rise to fine tonic movements; (vi) a deteriorated or immature cortex responds tonically; (vii) a single muscle of the forelimb may be activated phasically by the cortex in spite of the fact that all other muscles of both forelimbs and the skin of the region are completely denervated.

Considering the first and second observations together it would seem

unreasonable to suppose that phasic movement depends upon alternate activation of the points of cortical representation of flexion and extension at the cortical level. On the contrary, both observations are in favor of a uniform steady outflow to both flexors and extensors which is somehow converted at the spinal level and by afferent impulses into an alternating discharge. That it is unnecessary to consider afferent impulses from opposing muscular action as a factor in this fundamental segmental arrangement is indicated by the seventh observation and that it is not simply the result of kinesthetic stimulation is indicated by the first two observations.

That the mechanism for phasic reaction is essentially a fundamental pattern of cord morphology would seem to be an inescapable conclusion. That isolated spinal cord segments are capable of rhythmic activity is, of course, a commonplace but it is also true that very forceful and rapid phasic activity may be produced by capsular stimulation after ablation of the cortex, by thalamic irradiation in animals from which the motor cortex has been out for months and by the bursts which occur in the "rage"-like reactions of chronic thalamic animals. Besides the second stage of epileptic seizures is clonic and although such seizures are believed to be of cortical origin the responsible impulses do not travel in the pyramids. Possibly the corticospinal system should be looked upon as a mechanism superimposed upon this fundamental pattern in such a fashion as to interdigitate with and supplement it without actually entering into its basic arrangement. That this relationship is complex and must to some extent reflect myologic arrangement is shown by the gross nature of the deformation of the functional pattern which is produced by interference with a portion of it. That the pattern is more easily deformed upon the extensor than flexor side (iv above) would seem to be, in some measure, a reflection of the greater functional value which the flexor system possesses in an animal such as the cat which depends for its defense and food upon the flexor movements of clawing, climbing and catching (with its paws). We may ask whether, by the term "greater functional value," we are to understand greater flexor strength, more extensive flexor innervation or both? Probably the two situations are mutually interdependent. At one period of the work we thought that the fibers dealing with extension might travel in the most lateral third of the pyramid whereas flexion was represented throughout it but we were not able to convince ourselves of the validity of this observation. Whether they are specifically localized or not, however, they seem to be far less numerous than the flexor fibers.

Observations five and six seem to be essentially related with two and three. Apparently when the motor area is widely activated either by a "broad" stimulus or by means of its own intracortical association mechanisms its normal response is a widespread, steady, intraspinal activation of the limb area involved. The intrinsic spinal mechanisms convert this into phasic activity. In cases in which there is a defect in this widespread pyramidal discharge (due to selective stimulation, death or immaturity of cortical neurons or interruption of their axons) the intraspinal mechanism is not

called into play. The nature of the defect which occurs, when only cortical dissociation appears, is difficult to determine since both flexion and extension are still separately elicitable but not fusible. There is, moreover, a change in the cortical pattern of the excitable points. We have not been able to come to any conclusion about the nature of this (the question is under further investigation) since our series of animals was not designed for that purpose but a shifting in the cortical pattern is also seen in cases in which all the muscles but one of a limb are denervated (the cortical area capable of exciting this muscle becoming progressively larger as other muscles are rendered non-functional.) Again in strychnine poisoning, as is well-known, the entire motor cortex may become an extensor representation. All these observations tend to render the old statement that "not muscles but movements are represented in the cortex" about as meaningless as could be imagined and add to the difficulty in comprehending how the suprasegmental pattern is superimposed upon the fundamental, spinal, phasic mechanism. Whether or not this phasic mechanism will be activated seems to be determined then by two factors, first the activity of a large number of corticospinal fibers and second by the integrity of the intrinsic spinal mechanism. Thus the incoming kinesthetic impulses from a contracting muscle are not enough to produce phasic movement as we know from extrapyramidal stimulation. They must encounter a certain spinal, balanced discharge-reserve, one of the means of the establishment of which is relatively full pyramidal activity. The kinesthetic impulses may therefore be looked upon as tipping this balance of discharge-reserve now in one direction and now in another.

It is interesting to compare the effects of extrapyramidal stimulation with those obtained from corticospinal activation. In the former case a phasic discharge is rare (Mettler, 1940) and one wonders if it would not be useful to look upon any given extrapyramidal system as a partial motor unit. If two oppositional extrapyramidal units are simultaneously activated, just out of phase with each other, rather clumsy, phasic movements can be produced such as swinging of the head from side to side or a curious, serpentine wriggling of the spinal column. One of the difficulties in the attempt to produce nice, discriminating, phasic movements of this type is that most extrapyramidal units respond slowly and build up to a maximum which again breaks down slowly. Even in self-originated, extrapyramidal movements this is seen. Thus the decorticated dog, as previously reported (Mettler, Mettler and Culler, 1935), has considerable difficulty in starting and stopping movements. The sudden origination of an "upper level" movement is certainly a function of the pyramidal tract but we have no evidence that inhibition follows this course. Since inhibition is the subject of another communication we may dispense with its discussion for the present.

The functional importance of adequate sensory activity in determining the character of motor responses can hardly be overemphasized. Not only is good sensory activity important at both the cortical and spinal levels in pyramidal function but it also enters into the manifestations of extrapyram-

idal activity as well. Thus in the course of recent experiments with bulbo-
capnin we observed that deafferentation of the forelimb abolishes any evidence
of the action of the drug in the extremity concerned.² Again deafferentation
is not without an effect even in such an impelling condition as strychnine
poisoning.

The fact that movements and particularly phasic movements are pos-
sible in the deafferented animal might be superficially supposed to remove
from the realm of clinical significance such phenomena. Still the experienced
neurosurgeon knows that patients will not use a limb so affected if the in-
volvement is extensive and that in all cases it produces, to some degree,
an effect which amounts, for practical purposes, to paralysis. The phasic
movements seen in the usually flail-like deafferented extremities of our cats
were not of a pyramidal type and, in fact, it was pyramidal function which
was most seriously affected. Although such animals would finally succeed
in removing, with the anesthetic limb, an empty can placed over their heads
the movements were predominantly of the diffuse spinal type seen in the
sort of struggling the decorticated animal exhibits. In such cases a tonic,
dyskinetic element previously undetected would crop out in overflexion and
extensor thrusts which but for a temporary hypertonia were suspiciously
choreiform. In itself this seems relatively unimportant. The important point
is that in the clinic a neural dysfunction is not ordinarily seen, as it may be
revealed in the laboratory. What is seen is the subject's reaction to the
basic dysfunction which is often effectively hidden away under a rather
bizarre syndrome. The distinction between original dysfunction and com-
pensation for this should always be attempted in spite of the difficulties
involved.

CONCLUSIONS

1. The conversion of a cortically induced phasic movement into a tonic
movement by dorsal root section is a true neural effect and is not related
with (a) acute conditions, (b) the route by which the dorsal roots are ap-
proached nor (c) vascular damage.

2. The conversion of a cortically induced phasic movement into a tonic
movement by lesions placed in the "position of the ventral spinocerebellar
tract" as previously reported is the result of partial damage to the pyramidal
system and phasic activity is not converted into that of a tonic type by (a)
damage done to the spinocerebellar tract nor (b) rubrospinal tract elimi-
nation.

² Stimulation of the cortex of an animal given bulbo-
capnin, 40 mgm. per kg. subcut.,
sometimes gives tonic flexion but more usually phasic movements of a tremorous nature
are obtained. Epileptic seizures are easy to evoke. Deafferentation of the forelimbs of such
animals totally abolishes (in that region) the plastic tonus, which is characteristic of bul-
bo-
capnin poisoning. Such deafferented limbs do not respond to vestibular activation nor
do they display any tendency to cling to objects. Stimulation of the cortex of deafferented,
bulbo-
capnin-poisoned cats sometimes gives tonic flexion but more frequently loose, flabby
jerks of the flexor muscles alone is all that is obtained.

3 Cortically induced phasic movements furthermore cannot be converted into tonic movements by (a) removal of all nor any part of the cerebellum nor (b) destruction of the cerebellar nuclei

4 Cortically induced phasic movements may be obtained even though only the pyramids remain in connection between the medulla and cortex (though the medulla be entirely transected excepting for the pyramids)

5 Epileptiform seizures cannot be evoked from the cortex if only the pyramids are intact but can be evoked if they alone are severed Further the cortex of one hemisphere still retains its capacity for spinal inhibition after its corresponding pyramid has been severed

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FOREBRAIN AND RAGE REACTIONS

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INTRODUCTION

NEARLY a half a century has passed since Goltz (1892) in his classic account of a decorticate dog described the onset of abnormal emotional hyperexcitability, the animal being provoked to a marked reaction of anger and rage even by slight and innocuous stimuli. These observations were confirmed by the majority of later investigators, Rothmann (1923) on a decorticate dog, Dusser de Barenne (1920) and Bard and Rioch (1937) on decorticate cats, while negative results were reported only occasionally (Mettler and Culler, 1935). No one has ascertained, however, what part of the forebrain must be eliminated to cause the phenomenon. Whereas Goltz (1884) observed it after ablation of the frontal lobes and anterior sigmoid gyri, a number of observers (Barris, 1937, Magoun and Ranson, 1938) did not obtain these reactions even after extensive lesions of the frontal lobes. The experiments of Cannon and Britton (1935), Bard (1928), Bard and Rioch (1937) indicated that these fits of rage are due to the release of subcortical ganglia from cortical inhibition, particularly a release of the posterior hypothalamus as far as the vegetative component of these reactions is concerned, the somatic component probably having its center in the mesencephalic tegmentum and its continuation into the hypothalamus (Hinsey, Ranson and McNattin, 1930.)

A systematic study of the problem of rage reactions, therefore, must deal with all those parts of the forebrain which send efferent impulses to the hypothalamus and the midbrain tegmentum respectively. The following systems require consideration.

1. *Frontal lobe.* The precentral region and area frontalis agranularis respectively receive impulses from and send fibers (Fig. 1, 1a) to the anterior nucleus of the thalamus (Gruenthal, 1939). The Vicq d'Azyr bundle seems to contain not only fibers from the mammillary body to the anterior nucleus, but also fibers of conduction in the opposite direction (LeGros Clark, 1932). Impulses from the anterior nucleus may reach the hypothalamus also by way of the strio-pallidum, since degenerations from the anterior nucleus to the caudate nucleus have been observed (E. Sachs, 1909). In monkeys degenerating fibers (Fig. 1, 1b) have been traced from the precentral gyrus to the septohypothalamic nuclei and to the periventricular region (Mettler, 1935). After injury to the white matter of the frontal pole in a guinea pig Wallenberg (1934) traced a tractus neocortico-septalis (Fig. 1, 1c) to the dorsofrontal part of the septum pellucidum. The septal nuclei send a septo-hypothalamic tract joining the medial forebrain bundle to the hypothalamus (Loo, 1931). Also in experiments in monkeys Mettler (1935) found that

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prefrontal lesions gave rise to degeneration of fibers that could be traced to the septum. Mettler (1935) also traced in the monkey fibers from area 9 of the frontal pole into the corpus striatum and the subthalamus. According to this author, the medial nucleus of the thalamus receives fibers from the region anterior to the precentral gyrus. The medial nucleus of the thalamus may be connected with the hypothalamus by periventricular fibers.

2. *Parietal lobe.* Cortico-subthalamic fibers (Fig. 1, 2) arise, according to Mettler's (1935) experiments in monkeys, from the anterior and posterior

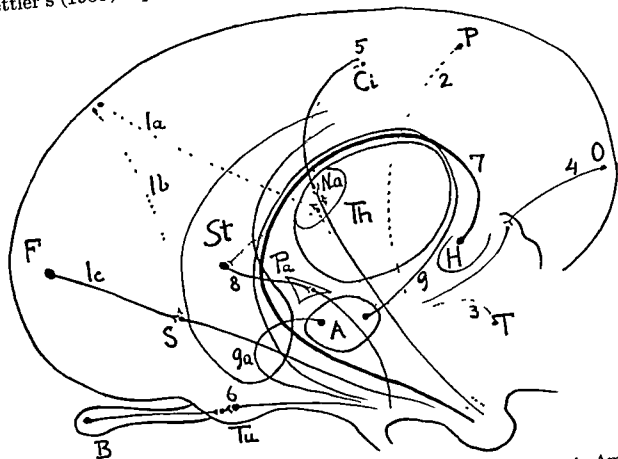


FIG. 1. Corticofugal systems to the hypothalamus and subthalamus A, Amygdala, B, Olfactory bulb, Ci, Gyrus cinguli, F, Frontal lobe, H, Hippocampus, Na, anterior nucleus, O, Occipital lobe; P, Parietal lobe, Pa, Pallidum, S, Septum, St, Striatum, T, Temporal lobe; Th, Thalamus, Tu, Olfactory tubercle (For further details see text)

marginal gyri and probably also from the medial parietal cortex. Probst (1903) traced degenerating fibers from the parietal lobe to the anterior nucleus of the thalamus in dogs and cats; the regio centralis, however, did not escape injury.

3. *Temporal lobe.* Degenerating fibers were followed from the lateral surface to the temporal lobe (Fig. 1, 3) to the subthalamus and to the lateral hypothalamic nuclei (Mettler, 1935). From the anterior end of the occipito-temporal gyrus and from the upper end of the superior and middle temporal gyrus, cortico-subthalamic fibers seem to originate (Mettler, 1935).

4. *Occipital lobe.* Centrifugal fibers from the occipital lobe end in the tectum of the superior colliculi (Fig. 1, 4). From here a system arises that

could be followed into the subthalamus (Forel's field H_1 , H_2) and zona incerta, Godlowski (1936).

5. *Gyrus cinguli*. Although the connection between the corpus mammillare and the anterior nucleus of the thalamus and the gyrus cinguli is mainly cortico-petal (Papez, 1939), the possibility of the existence of inhibitory influences from the gyrus cinguli through the anterior medial thalamic nuclei to the hypothalamus (Fig. 1, 5) has to be considered (Grinker, 1939). Fibers of the fornix longus perforating the corpus callosum may also represent a connection between medial parts of the cerebral cortex and the base of the diencephalon (Obersteiner, 1912).

6. *Olfactory tubercle*. (Fig. 1, 6) This structure sends impulses to the closely adjacent cells of the dorsally situated motor olfactory striatum (Papez, 1929) which lie ventral to the head of the caudate nucleus proper. Fibers originating in the motor olfactory striatum join the medial forebrain bundle and descend to the hypothalamus (olfactory peduncular tract of Papez). Also fibers from the pyriform lobe join this bundle. Fibers from the secondary olfactory centers in the pallium may also reach the hypothalamus by way of the lateral forebrain bundle (Kuhlenbeck, 1927).

7. The *hippocampus-fornix* system (Fig. 1, 7) to the mammillary body and to the tuber is well defined (see Ingram, 1940) and requires no further description.

8. The *striatum-pallidum* system (Fig. 1, 8) is continued by pallidofugal fibers which end in the tuber.

9. The *amygdaloid nuclei* give rise to the stria terminalis (Fig. 1, 9), the fibers of which are distributed to most major hypothalamic nuclei (Kappers, Huber and Crosby, 1936). There exist in addition (Fig. 1, 9a) fibers from the lateral group of the amygdaloid nuclei which have no connection with the stria terminalis but are connected with the preoptic and hypothalamic area by fibers joining the anterior commissure (Humphrey, 1936).

MATERIAL AND METHODS

The results reported in this paper are based on observations on the spontaneous general behavior and emotional excitability of 66 cats in which the various systems described above were eliminated in acute experiments; twelve dogs were used in which extirpation of the frontal lobes, of the lateral surface of the parietal and temporal lobes had been performed for other purposes, the animals having survived for from four weeks to over one year.

The cats were operated under ether anesthesia and the dogs under nembutal anesthesia. In the experiments in which large parts of the hemispheres were removed (*e.g.*, lobectomies performed) ligature of the carotid arteries preceded the brain operation. Lesions of the subcortical ganglia or tracts were usually made by electrolysis with the aid of Horsley-Clarke's stereotaxic apparatus. In a few cases the ablations were performed in two stages, in order to ascertain whether one sided elimination of the systems under study was sufficient. Since these unilateral operations gave inconclusive results, in the majority of the experiments bilateral lesions were placed in a one stage operation.

RESULTS

In the acute experiments on cats, the animals in which positive results were obtained, *i.e.*, "rage positive" animals, exhibited an abnormally in-

creased motor activity. This appeared in some animals immediately upon awakening from the ether anesthesia, in others after a latent period covering a few up to 45 min. Restlessness was especially apparent when liberty of movement was restricted, *e.g.*, by keeping the animal in a small cage. Thus confined, it would go through recurrent fits of violent activity at intervals of five to fifteen minutes. Often it pushed against the wall or corner of the cage. Frequently it was seen struggling with its forepaws or trying to jump against the lid of the cage. These movements were accompanied in typical cases by clawing, waving of the tail, hissing or biting. The abnormal excitability was promptly apparent if one tried to remove the animal from its cage or to raise it by the back of the neck. The animal would hiss, sometimes try to bite and claw or jump into the air.

When such animals were left in the room without restraint the fits were less marked. They exhibited, however, a tendency to run about rather aimlessly, to press head-on against any resistance they encountered, and to withdraw into corners. Sometimes the cats would jump almost vertically upward.

Polypnea was striking and observed often directly after operation even before recovery from ether anesthesia. The increased respiratory rate, up to 150 per min., sometimes greater, often became especially prominent during the fits of motor excitement, and often outlasted the motor component of the fit. The exhausted animal would fall into a recumbent position, the polypnoea continuing. The picture of the motor component of the rage reactions as here described is not always developed in all its elements. We considered, however, an experiment as rage positive only if several of the above described symptoms appeared combined; the appearance of a single component, *e.g.*, restlessness, did not seem to justify the diagnosis of rage reaction.

It should be emphasized that whereas these motor outbursts were often accompanied by signs of hyperactivity of the vegetative nervous system, *e.g.*, pupillary dilatation, piloerection, often there occurred a dissociation of motor and vegetative manifestations. For example, as nearly as we could judge by simple observation, there were occasions when no evidence of vegetative discharge seemed to accompany the motor outbursts and polypnoea. It is, of course, possible that recording devices might have captured vegetative disturbances like vasoconstriction which escaped our powers of gross observation.

As to chronic symptoms, witnessed in dogs, they were similar to those described by Goltz (1892): overexcitability, barking, baring of the teeth, attempts to bite, etc. if one approached the animal or tried to lift it from the cage.

Experimental elimination of the following regions failed to produce outbursts of rage reactions: sigmoid and coronal gyri, lateral surface of the parietal lobe and the gyrus marginalis on its medial surface, lateral surface of the temporal lobe, lateral as well as medial surface of the occipital lobe.

The frontal poles (frontal lobes in front of the sigmoid gyri) were destroyed in eight cases, either alone or in combination with the motor cortex, with the medial surface of the frontal lobe, with the gyrus rectus and with the tip of the head of the caudate nucleus respectively. Definite outbursts of rage failed to appear in seven of these eight cases; five of them showed a more or less marked hypermotility as was previously observed by



FIG. 2. Cat 66. Bilateral lesion of the olfactory tubercles. 5/24/40 11:00 A.M.-12:10 P.M. Ether anesthesia. Ligature of both carotid arteries. Cauterization of both olfactory tubercles on the overhanging brain. 12:50-4:00 P.M. Repeated spontaneous outbursts of hypermotility, pushing against corners of cage, struggling. Respiratory rate up to 60 per minute. Excitement and struggling are increased if one takes the animal from the cage and holds it by the back of the neck.

Magoun and Ranson (1938) and Langworthy and Richter (1939). This hypermotility by itself did not seem to warrant the inference that we were dealing with rage reactions. In one animal there were definite outbursts of rage. In this case the lesion encroached not only upon the head of the striatum but also upon the white matter dorsal to the olfactory tubercle so that the change in behavior could hardly be ascribed to the frontal pole lesion alone. This inference received further support from three subsequent experiments in which positive reactions were obtained. In these three animals the frontal lobectomy was performed in such a manner that the level of the dorsal entry of the section (ansate sulcus) corresponded with that of the preceding experiments. The ventral end of the section on the base, however, instead of being anterior to the olfactory tubercle, lay on the anterior border or a few millimeters in front of the optic chiasma.

In these animals with bilateral frontal lobectomy in which expressions of rage appeared, a peculiar association with cataleptic reactions could be observed. After stopping their running movements or struggling, the animals sometimes assumed peculiar postures for several minutes. This cataleptic reaction resembled that described by Barris follow-

ing the bilateral removal of the rostral part of the neocortex in cats, the animals showing no rage reactions.*

These experiments directed our attention to the base of the brain, particularly to the olfactory centers. Using Karplus and Kreidl's method of operation on the overhanging brain the olfactory bulbs were destroyed on both sides by thermocautery, or their stalks were sectioned. While these operations produced no or only slight and transitory effects, lesions encroaching upon the olfactory tubercles or isolated lesions of the tubercles (Fig. 2) were followed by distinct rage reactions (6 cases), particularly if the lesion extended medialward to the septum pellucidum.

* The appearance of such cataleptic symptoms, however, seems not specifically related to the frontal lobes. Occasionally it could also be found in animals with other lesions, e.g., bilateral injury to the amygdaloid nuclei.

Lesions of the hippocampus-fornix system were produced by electrolysis with the help of the Horsley-Clarke apparatus (Fig. 3); or the corpus callosum was exposed and incised, and the lesions were placed in the hippocampus by electrocautery, or the fornices were cut before they descended into the thalamus. Of seventeen cases in which acute lesions of this system were made, four did not show manifestations of rage (#45, unilateral lesion of the hippocampus, #44 puncture of the fornices, #43 and #47, puncture of the commissura hippocampi). In three cases (#6, 34, 54) there was a certain restlessness, but without definite rage reactions. Ten experiments could be considered rage positive showing either the full developed picture of rage or at least rudimentary reactions (e.g., vocal expressions of rage). In three of these ten cases the lesion encroached upon the most dorsal part of the thalamus (#16, 17) and the stria medullaris (#40), respectively, in four others upon the Septum pellucidum immediately in front of the fornices (#41, 42, 45, 55). To this latter group belong animals (#41, 55) with very marked rage reactions.

Since a longitudinal section of the corpus callosum had to be performed in order to expose the hippocampus, and since a part of the callosal fibers and of the gyrus cinguli had to be punctured in placing the electrolytic lesions, control experiments with lesions of these parts were necessary. Median section of the corpus callosum and also puncture of the gyrus cinguli with the needle of the Horsley-Clarke apparatus failed to produce rage reactions. In seven cats extensive lesions of the gyrus cinguli were performed by electrocautery. Four of these experiments were rage negative. In one case (#35) in which definite outbursts of rage appeared, there was a slight additional injury to the hippocampus; in a second animal (#37) showing rage a small cotton sponge had been left inadvertently in the operative cavity apparently pressing upon the underlying corpus callosum and hippocampus. Finally, in one cat (#20) in which bilateral frontal lobectomy had failed to produce changes in emotional behavior, additional extirpation of the gyri cinguli was followed by transient and weak outbursts of rage. The lesion in this animal reached deeply into the white matter close to the head of the caudate nucleus. Thus, the influence of the gyrus cinguli upon the subcortical mechanisms responsible for manifestations of rage is hardly very pronounced, while an influence of the hippocampus-fornix system is more definite. It should, however, be admitted that rage symptoms may be enhanced, if the lesion of the fornices



FIG 3 Cat 41 Electrolytic lesion of the fornices and the septum pellucidum 2/7/40 10 45-11 50 A M Electrolytic lesion under ether anesthesia 12 00-4.30 P M Very marked restlessness and excitement Hissing, clawing and jumping against the lid of the cage, tries to bite when approached Respiratory rate up to 120 per minute

is combined with a lesion of centrifugal fibers from the gyrus cinguli. This is suggested by the observation that the outbursts of rage were particularly pronounced in cases in which the lesion of the fornices encroached upon the septum pellucidum, which contains fibers of the fornix longus from the gyrus cinguli to the base of the brain. A concomitant lesion of the neocortico-septal tract may, however, also play a part in these cases, *e.g.*, as far as the production of the hypermotility is concerned, since ablation of the frontal poles may cause a certain restlessness (see above).



FIG. 4. Cat 48. Bilateral electrolytic lesion of the amygdaloid nuclei 2/27/40 11:30-12:30 P.M. Electrolytic lesion under ether anesthesia. 12:40-3:50. Nearly continuous hypermotility, tendency to circus movements (clockwise), pushing against the walls and corners of the cage, jumping against the lid and clawing, struggling, howling. The outbursts of excitement are often followed by catatonic postures.

After bilateral electrolytic lesions had been produced in the head of the caudate nucleus (sparing the motor olfactory striatum), the emotional excitability of the cats was not increased. The same result held true for bilateral lesions which extended to the tail of the caudate nucleus. Definite outbursts of rage reactions appeared after bilateral electrolytic lesions of the oral part of the amygdaloid nuclei (Fig. 4). Control experiments showed that the animals remained quiet after punctures at the same coordinates of the Horsley-Clarke apparatus provided the lesions remained dorsal to the amygdaloid nuclei. In view of the positive results from amygdaloid lesions one would presuppose that bilateral section of the stria terminalis would give similar results. Such, however, was not the case. This failure is probably due to the fact that the stria terminalis is not the sole efferent pathway of the amygdaloid ganglia, as pointed out in the introductory anatomical remarks. The possibility must of course be borne in mind that the lesions of the amygdaloid nuclei also affected efferent fibers of the pyriform lobe. Lesions of the pyriform lobes produced by thermocautery on the overhanging brain, however, evoked only slight and transient symptoms of rage as long as they remained superficial.

COMMENT

The fact that injury of the olfactory tubercles is followed by rage reactions may explain why some authors observed such reactions after extirpation of the frontal lobes (Goltz, 1884) while other authors who removed

neocortical parts only (Barris, 1937; Magoun and Ranson, 1938; Bard, 1939) failed to elicit such changes of behavior. The appearance of these phenomena after incision at the base of the brain a few millimeters in front of the optic chiasma (Fulton and Ingraham, 1929) seems to be due mainly to section of the descending fibers from the olfactory tubercles (perhaps also fibers from the septal nuclei and fibers from the amygdaloid complex that first enter the anterior commissure and thence pass caudalward).

The experiments presented in this communication indicate that rage reactions appear with lesions of the olfactory tubercles, of the amygdaloid nuclei and to some extent also with lesions of the hippocampus-fornix system. These parts of the forebrain, all phylogenetically old, enter into the formation of the central olfactory system. The appearance of these reactions, however, cannot be explained simply on the basis of elimination of afferent olfactory impulses as shown by a comparison with the effects of extirpation of the olfactory bulbs. In this connection it is pertinent to recall that not only the olfactory tubercle and the amygdaloid complex (Edinger, 1911; Spiegel, 1919) but also the hippocampus (LeGros Clark, 1932) are rather well developed in micro- and anosmatic mammals. This had raised the thought that these structures may also subserve non-olfactory functions (Edinger, 1911; Herrick, 1933; Papez, 1937).

In connection with our problem the views of Herrick (1933) and Papez (1937) possess a special interest. The former assumes the existence of activating impulses from the olfactory cortex upon the cerebrum, influencing affective tone. According to the concept of Papez, hippocampo-fugal impulses acting upon the mammillary bodies and thence upon the gyrus cinguli play an important role in the mechanisms of emotive processes. This in turn provokes the question whether the changes in behavior following decortication are only symptoms of release due to the loss of cortical inhibition or whether stimulation of excitatory systems also plays a part.

It is well known that the cortex may send stimulating, as well as depressing impulses, to a certain system such as the vasomotors, for instance. The occurrence of rage symptoms for several months after decortication or after prechiasmal punctures (Fulton and Ingraham, 1929) shows, of course, that following these procedures a certain overexcitability of subcortical mechanisms responsible for bodily manifestations of rage may develop as a result of the loss of inhibitory impulses or as an isolation phenomenon. This does not exclude the possibility, however, that corticofugal systems may exist which have an excitatory or facilitating influence upon the subcortical apparatus innervating rage reactions and that stimulation of such corticofugal systems may contribute to the genesis of the manifestation of rage in the acute preparation.

On stimulation of the rostral pyriform area Rioch and Brenner (1938) observed struggling and biting. By stimulation of the subfornical component of the medial forebrain bundle Ranson and Magoun (1933) elicited motions of spitting, while stimulation of the preoptic region, the medial forebrain

bundle or of the fornix in the rostral part of the hypothalamus failed to produce respiratory acceleration, running movements or dilatation of the pupils, reactions which are observed upon hypothalamic stimulation. Yet the existence of excitatory or facilitating corticofugal impulses from the hippocampus is suggested by the experiments of Klüver and Bucy (1939). In accordance with the theory of Papez, ablation of the temporal lobes with destruction of the hippocampi was followed in their chronic experiments* by a diminution of reactions of fear and anger and only with regard to sexual reactions was the emotional activity increased. Thus, it seems not improbable that stimulation of corticofugal fibers has its place in the genesis of the rage reactions in the acute preparation.

It will hardly be surprising to find differences in the effect of forebrain lesions in carnivores on the one hand and in monkeys and men on the other. The cortical representation of the mechanisms of emotional expression may assume a greater significance as one ascends the phylogenetic scale (Fulton, 1939). Furthermore a comparison with observations on monkeys after lesions of the frontal lobes (Jacobsen, 1931; Kennard and Ectors, 1938; Richter and Hines, 1938) and with clinical experiences (Davison and Kelman, 1939) with forced laughing and crying associated with lesions of the neocortex suggests the possibility that with progressive encephalization parts of the neocortex develop an inhibitory influence upon the subcortical mechanisms responsible for affective reactions. Such an influence is exerted in the more primitive brain of carnivores chiefly by phylogenetically old parts of the forebrain.

SUMMARY

A study was made of the parts of the forebrain which are related to the initiation of rage reactions observed in decorticate cats and dogs. Lesions restricted to neocortical areas failed to produce rage reactions. Following lesions of the frontal poles hypermotility could be observed but no convincing outbursts of rage. Definite manifestations of rage appeared if the lesions (e.g., extirpation of the frontal lobes) encroached upon the olfactory tubercles or followed isolated lesions of the tubercles, whereas destruction of the olfactory bulbs or section of their stalks had no or only slight effects. After acute lesions of the hippocampus-fornix system rudimentary, or in some animals marked, rage reactions appeared, particularly in cases in which the lesion of the fornices encroached upon the septum pellucidum. Definite outbursts of rage were observed after bilateral lesions of the amygdaloid nuclei. Lesions of the pyriform lobes, as long as they remained superficial, evoked only slight and transitory symptoms of rage.

* Klüver & Bucy operated upon their monkeys under sodium pentobarbital anesthesia, so that the animals slept immediately following the operation, while our acute experiments on cats were performed under ether anesthesia so that the acute stage following the operation could be observed as in Bard's acute experiments.

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